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(71) Applicant: GENENOVA CORPORATION [US/US]; 1533–34th Avenue, San Francisco, CA 94122 (US).

(72) Inventors: LIAO, Jaw–Ching; 3F No. 3 25 Valley, Chung–Cheng Road, Pan–Chiao, Taipei (TW). WANG, Cheng–Nan; 12th floor, No. 242, Kuang Fu South Road, Taipei (TW).

(74) Agents: KING, Joshua et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104–7092 (US).

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(54) Title: DEVICES AND METHODS COMPRISING A SERUM ALBUMIN – TARGET ANTIGEN COMPLEX

(57) Abstract

Improved methods and compositions for the detection and diagnosis of disease utilizing a target antigen complexed with a serum albumin to provide a conformationally changed target antigen. Also, antibodies, including both IgM and IgG antibodies as well as all other forms of antibodies, specific for serum albumin and/or for the conformationally changed target antigens, as well as methods for purifying each using immunologic methods such as affinity columns. Enhanced methods and compositions comprising a target antigen complexed with a serum albumin for the induction of immune responses in an animal, as well as enhanced vaccination of such an animal. Further, a preferred buffer, TNSCN, comprised of Tris–HCl, NaCl, and NaSCN, typically at a pH of about 7.4 ± 0.2.

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DEVICES AND METHODS COMPRISING A SERUM ALBUMIN – TARGET ANTIGEN COMPLEX

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/730,912, filed October 18, 1996, now pending.

TECHNICAL FIELD

The present invention relates generally to antibodies against a serum albumin, and to methods and compositions for the highly specific, highly sensitive detection of a target antigen that can associate with a serum albumin or antibodies against such a target antigen. The methods and compositions are also suitable for the elicitation of an immune response in an animal, and for the vaccination of an animal, against the target antigen.

BACKGROUND OF THE INVENTION

Serum albumin is a small, relatively symmetrical protein with a calculated molecular weight between about 65,000 and 70,000. Serum albumin functions as a principal protein in plasma, and it has a relatively low intrinsic viscosity and a strong internal structure held together by disulfide bridges. Tullis, J., *J.A.M.A. 237*(4):355-360, 1977; Tullis, J., *J.A.M.A. 237*(5):460-463, 1977.

In its native state, serum albumin is a highly soluble molecule carrying a strong net negative charge, and an isoelectric point between about pH 5.4 and 4.4. Despite the high net negative charge of serum albumin, it can bind reversibly to both cations and anions, making it suitable for the transport or inactivation of substances such as trace metals, drugs, dyes, fatty acids, hormones, and enzymes. *Id.* Principally, albumin has been used in clinical applications for its oncotic action as a plasma volume expander. *Id.* Serum albumin has been believed to lack either enzymatic or immunological functions. Yeh, P. et al., *Proc. Natl. Acad. Sci. USA 89*:1904-1908, 1992. Thus, there is a need for methods and products that assist in the manufacture of serum albumin.

Turning to another issue, a wide variety of diseases, such as infectious diseases, require effective diagnostic assays, adjuvants that enhance the effect of vaccines, and vaccines. Examples of such infectious diseases include acquired immune deficiency syndrome (AIDS) which is caused by a group of retroviruses known as HIV (Barre-Sinoussi et al., *Science 220*:868-871, 1983; Gallo et al., *Science 224*:500-503, 1984; Coffin et al., *Science 232*:697, 1986) and the hepatitis C virus (HCV) (Choo et al., *Science 244*:359-362, 1989).

HIV-1 is typically transmitted by sexual contact, by exposure to blood or certain blood products, or by an infected mother to her fetus or child (Piot et al., *Science 239*:573-579, 1988). Examples of transfusion-associated HIV-2 infection have also been disclosed (Courouce et al., *AIDS 2*:261-265, 1988). Similarly, HCV is often transferred via blood transfusion; post-transfusion hepatitis (PTH) occurs in approximately 10% of transfusion patients, and HCV (*i.e.*, Non-A, Non-B hepatitis (NANBH)) accounts for up to 90% of these cases.

With regard to HIV, EIAs based on whole virus or viral lysate have been developed for detection of the virus. However, it has been found that the EIAs have undesirable, non-specific reaction with specimens from individuals with non-HIV conditions such as autoimmune diseases, a history of multiple pregnancies, anti-HLA, EBV infections or hypergammaglobulinemia. Also, an ELISA has been developed and commercialized by Abbott Laboratories for serological diagnosis of HIV infection using the HIV-1 core and HIV-1 envelope and HIV-2 envelope proteins. However, these assays have not provided the highly specific, highly sensitive detection needed for superior protection of the blood supply, or for early diagnosis of HIV in a patient.

With regard to HCV, a specific assay using an HCV antigen designated C100-3 was created using recombinant DNA methods in yeast (*Science 244*:362-364), and an enzyme-linked immunosorbent assay (ELISA) was developed for serological diagnosis of hepatitis C virus (HCV) infection using the HCV core protein (p22). The core protein was synthesized by a recombinant baculovirus (Chiba et al. *Proc. Natl. Acad. Sci. USA 88*:4641-4645, 1991). However, these assays failed to detect a

significant number of cases of HCV infection, even when relatively large sample volumes were available.

Turning to the development of adjuvants that enhance the immunoinductive effect of vaccines against pathogenic agents such as HIV and HCV, as well as such vaccines themselves, at present, alum (aluminum hydroxide and aluminum phosphate) is the only adjuvant approved for vaccines in the United States. Alum has a history of successful use with low toxicity (*AIDS Research and Human Retroviruses 11*, 1995), but the need for other adjuvants having one or more superior properties has gone unmet. The provision of alternative adjuvants would provides users with a variety of strategies, and the ability to select and choose between superior qualities based upon particular needs. *See Vaccines and World Health 227*, Oxford, 1994; Mosby, *Immunology*, 4th ed., p. 19.8-19.9; Mosby, *Clinical Immunology, Principles and Practice*, Chapter 123, p. 1911-1912.

Thus, there has gone unmet a need for products and methods capable of highly specific, highly sensitive detection of pathogenic agents. There has also gone unmet a need for products and methods capable of eliciting, or enhancing the elicitation of, an immune response to such pathogenic agents, especially an immunoprotective immune response to such pathogenic agents. The present invention utilizes properties of serum albumin and target antigens associated with pathogenic states to provide these advantages, as well as other related advantages.

SUMMARY OF THE INVENTION

The present invention is directed to the use of serum albumin, including human serum albumin, as an active component of the immune response. The serum albumin aggregates with antigens to provide a complex comprised of invading antigen(s) and serum albumin(s). Pursuant to such complexing, the invading antigen is believed to undergo conformational changes that affect the antigenicity of the antigen. This complexed antigen is then recognized by the immune system, which produces antibodies (such as IgG and IgM antibodies) that have a high specificity and affinity for the complexed antigen, although such antibodies typically do also bind the

uncomplexed antigen to a lower specificity and affinity. Additionally, perhaps because the serum albumin is maintained in such close proximity to the invading antigen, the immune system produces antibodies against the serum albumin itself.

The discovery of this advantage found with a complexed antigen permits the design of assays, methods and compositions that are directed toward the detection of the complexed antigen and the use of the complexed antigen, and the detection and use of antibodies to such a complexed antigen. Such discovery also permits the design of compositions and methods for the induction of an immune response in an animal, including an immunoprotective immune response. The present invention also provides for the provision of anti-serum albumin antibodies, as well as the detection of a diseased state by the detection of anti-serum albumin autoantibodies in a sample. In addition, the provision of anti-serum albumin antibodies or autoantibodies to a patient may interfere with the interactions between the serum albumin and the invading infectious agent, thereby depriving the infectious agent of an interim "host," which might otherwise assist the infectious agent in its invasion of the host animal. The antibodies and autoantibodies discussed throughout this disclosure include all forms of antibodies, and in preferred embodiments comprise IgG and/or IgM antibodies.

Accordingly, in one aspect the present invention provides an assay for the detection of an antibody against a target antigen in a sample, comprising: (a) providing a target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex; (b) contacting the target antigen-serum albumin complex with the sample under conditions suitable and for a time sufficient for the target antigen-serum albumin complex to bind to one or more IgM and/or other forms of antibodies for the target antigen present in the sample, to provide an IgM and/or other forms of antibody-bound complex; and, (c) detecting the IgM antibody-bound complex, and therefrom determining whether the sample contains the antibody for the target antigen.

The following embodiments apply to both the above aspects of the invention and other aspects of the invention, unless the context clearly indicates otherwise. For example, the complex can further comprise a second target antigen complexed with the target antigen-serum albumin complex, to provide a multiple target

antigen-serum albumin complex. In another embodiment, the assay further comprises the step of binding the target antigen-serum albumin complex to a solid substrate. Such binding to the solid substrate can be performed either before or after the step of contacting the target antigen-serum albumin complex with the sample.

In preferred embodiments, the serum albumin is selected from the group consisting of human serum albumin, bovine serum albumin, fetal bovine serum albumin, new born bovine serum albumin and mouse serum albumin. The sample can be either a purified or an unpurified sample, and the methods can further comprise, prior to the step of contacting, isolating the sample from an animal. The sample can be isolated from a human being, and the sample can be a blood sample. Typically, the serum albumin is selected from the same species as the sample, although the serum albumin can be from a different species, particularly for assay purposes.

The assays of the present invention can be selected from the group consisting of a countercurrent immuno-electrophoresis (CIEP) assay, a radioimmunoassay, a western blot assay, a radioimmunoprecipitation, an enzyme-linked immuno-sorbent assay (ELISA), a dot blot assay, an inhibition or competition assay, a sandwich assay, an immunostick (dip-stick) assay, a simultaneous assay, an immunochromatographic assay, an immunofiltration assay, a latex bead agglutination assay, an immunofluorescent assay, a biosensor assay, and a low-light detection assay.

In another aspect, the present invention provides a method of producing an antibody, such as an IgM or IgG antibody, specific for a target antigen, comprising the following steps: (a) administering to an animal the target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex under conditions suitable and for a time sufficient to induce the production in the animal of antibodies to the target antigen; and, (b) isolating the antibodies to the target antigen. The present invention also provides antibodies, such as IgM or IgG antibodies, produced according to such methods, as well as other antibody production methods disclosed herein. For example, the present invention provides an antibody specific for a target antigen, the antibody made against a target antigen-serum albumin complex comprising the target

antigen complexed with a serum albumin. In a preferred embodiment, the antibody is bound to a solid substrate.

In a further aspect, the present invention provides an assay for the detection of a target antigen in a sample, comprising: (a) contacting the sample with an antibody, such as an IgM or IgG antibody, produced according to the methods discussed above under conditions suitable and for a time sufficient for the antibody to bind the target antigen, to provide a bound antibody; and, (b) detecting the bound antibody , and therefrom determining whether the sample contains the target antigen.

In still a further aspect, the present invention provides an assay for the detection of an antibody specific for a serum albumin in a sample, comprising: (a) contacting the serum albumin with the sample under conditions suitable and for a time sufficient for the serum albumin to bind to one or more antibodies, such as IgM or IgG antibodies, specific for the serum albumin present in the sample, to provide an antibody-bound serum albumin; and, (b) detecting the antibody-bound serum albumin, and therefrom determining that the sample contains the antibody specific for the serum albumin.

In still another aspect, the present invention provides a method of producing an antibody, such as an IgM or IgG antibody, against a serum albumin, comprising the following steps: (a) administering to an animal a target antigen-serum albumin complex, comprising the target antigen complexed with a serum albumin, under conditions suitable and for a time sufficient to induce the production in the animal of antibodies specific to the serum albumin; and, (b) isolating the antibodies to the serum albumin. The method can also include the step of isolating the antibody from the animal. In a further aspect, the present invention provides an isolated antibody that is specific for a serum albumin, preferably human serum albumin. Such antibody can be bound to a solid substrate.

In still yet another aspect, the present invention provides a method for the detection of a serum albumin in a sample, comprising: (a) contacting the sample with an antibody, such as an IgM or IgG antibody, against serum albumin produced as described above under conditions suitable and for a time sufficient for the antibody to

bind the serum albumin, to provide a bound antibody; and, (b) detecting the bound antibody, and therefrom determining whether the sample contains the serum albumin. In an alternative aspect, the present invention provides a method for the isolation of a serum albumin from a sample, comprising: (a) contacting the sample with an antibody, such as an IgM or IgG antibody, against serum albumin produced as described above under conditions suitable and for a time sufficient for the antibody to bind the serum albumin, to provide bound serum albumin; and, (b) isolating the bound serum albumin from the sample.

The present invention also provides a method of producing an assay to detect an antibody, such as an IgM or IgG antibody, against a target antigen comprising contacting the target antigen with a serum albumin under conditions suitable and for a time sufficient for the target antigen to complex with the serum albumin to form a complex, and then binding the complex to a solid substrate. In a preferred embodiment, the target antigen is contacted with the serum albumin in a TNSCN buffer.

In yet a further aspect, the present invention provides a composition capable of binding to an antibody, such as an IgM or IgG antibody, against a target antigen, the composition comprising an isolated target antigen complexed with a serum albumin to provide an isolated target antigen-serum albumin complex. Preferably, the isolated target antigen-serum albumin complex is bound to a solid substrate. In an alternative aspect, the present invention provides a composition capable of binding to a target antigen, the composition comprising an antibody, such as an IgM or IgG antibody, produced against a target antigen-serum albumin complex comprised of the target antigen complexed with a serum albumin, the antibody bound to a solid substrate.

In still yet another aspect, the present invention provides a composition capable of eliciting an immune response in an animal comprising a target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex, in combination with a pharmaceutically acceptable carrier or diluent. In one embodiment, as discussed above, a second target antigen is complexed with the target antigen-serum albumin complex, to provide a multiple target antigen-serum albumin complex.

In still yet a further aspect, the present invention provides a method of enhancing the immune response to a target antigen associated with a pathogenic state, comprising the following steps: (a) complexing the target antigen with a serum albumin to provide a target antigen-serum albumin complex, whereby the serum albumin is an adjuvant; (b) combining the complex with a pharmaceutically acceptable carrier or diluent; and, (c) administering the complex with the pharmaceutically acceptable carrier or diluent to an animal, thereby providing an enhanced immune response to the target antigen.

In even yet a further aspect, the present invention provides a composition suitable for the treatment of a disease in an animal comprising a selected drug complexed with a serum albumin to provide a drug-serum albumin complex, in combination with a pharmaceutically acceptable carrier or diluent.

In even yet another aspect, the present invention provides a kit for the detection of a target antigen, the kit comprising: (a) the target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex; and, (b) one or both of a reagent or a device for detecting the complex or an antibody, such as an IgM or IgG antibody, bound to the complex. In an alternative embodiment, the kit for the detection of a target antigen: (a) an antibody (such as an IgM or IgG antibody), produced as discussed herein; and, (b) one or both of a reagent or a device for detecting the antibody.

These and other aspects of the present invention will become evident upon reference to the following detailed description. In addition, as noted above, various references are set forth throughout the present specification that describe in more detail certain procedures or compositions (*e.g.*, plasmids, etc.); all such references are incorporated herein by reference in their entirety.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that serum albumin in the body, including human serum albumin, is an important component of the immune response. It appears that the serum albumin in the body interacts with antigens

associated with an infectious agent, such as the core protein of Hepatitis C Virus, to provide a complex comprised of invading antigen(s) and serum albumin(s). Pursuant to such interaction, the invading antigen is believed to undergo conformational changes that affect the antigenicity of the antigen. This conformationally changed antigen is then recognized by the immune system, which produces antibodies that have a high specificity and affinity for the changed antigen, although such antibodies typically do also bind the conformationally unchanged antigen to a lower specificity and affinity. Additionally, when the target antigen is introduced in to an animal, the immune system produces antibodies against the serum albumin itself. Such antibodies may include all forms of antibodies, and in particular, IgM and/or IgG antibodies.

In accordance with this discovery, the present invention provides improved methods and compositions for the detection and diagnosis of disease wherein the assays utilize conformationally changed target antigens associated with a pathogenic state such as an infectious disease. The present invention also provides antibodies specific for serum albumin and antibodies specific for the conformationally changed target antigens, as well as methods for purifying each using immunologic methods such as affinity columns.

These significant advantages in antigenicity and epitopic configuration also provide enhanced methods and compositions for the induction of immune responses in an animal. These responses may be humoral, cellular or both. The present invention also provides enhanced vaccination of such an animal. Accordingly, the present invention features compositions and methods utilizing a serum albumin aggregated with a target antigen to provide a target antigen-serum albumin complex. The complex provides a synergistic effect when compared to the effect of target antigen alone, and will enhance the reaction of the target antigen with its corresponding antibodies at least about 1.1, and preferably at least about 1.25, 1.5 or 2.0 times more strongly when compared to the reaction of the antibodies with the target antigen alone, when the OD value is measured at 492 nm in a spectrophotometer.

The present invention also provides a preferred buffer, TNSCN, comprised of Tris-HCl, NaCl, and NaSCN, typically at a pH of about 7.4 ± 0.2. The

buffer facilitates the complexing between the serum albumin and a target antigen, and is useful in assays utilizing such complexing. The buffer is also useful other traditional immuno-based assays, such as ELISAs and RIAs, even if no complexing between targets is involved (for example because there is no molecule with which to complex).

The present invention also provides antibodies against serum albumin, methods of purifying serum albumin using such antibodies, and serum albumin so purified. These and other aspects and features of the present invention are discussed further below.

"Target antigen" is used herein to indicate a selected protein that is capable of inducing an immune response in an animal and is capable of being bound (typically at a particular epitope) by an antibody generated by the animal. The target antigen can be either recombinant or purified from a native source. Further, the target antigen is capable of forming a complex with the serum albumin, which complex, as discussed above, enhances the antigenicity of the target antigen. Determination of whether the target antigen can form such a complex with the serum albumin can be determined by mixing the target antigen with a serum albumin in a coating buffer such as TNSCN, TNEU, carbonate buffer, TNSDS or PBS, and then determining whether a dimer, trimer, multimer, or other oligomer has been formed comprising one or more target antigens and one or more serum albumins.

In a preferred embodiment, the target antigen is a protein associated with a pathogenic state, such as a cancer or an infectious or other disease. Further, preferably, the disease is a blood-borne infectious disease, so that antigens associated with the disease are presented while in the blood stream during normal infection, and therefore have the opportunity to interact with serum albumins during such infection. The infectious disease can be bacterial or viral, and in one preferred embodiment is a positive-stranded RNA virus such as Togaviridae, Coronaviridae, Retroviridae, Picornaviridae, Caliciviridae and Flaviviridae. Examples of such positive-stranded RNA viruses are discussed in U.S. Application Serial No. 08/447,276 and U.S. Application Serial No. 08/454,928. Examples of suitable target antigens include a core antigen of HCV, a core-envelope fusion protein from HCV (such as EN-80-2 (ATCC

55451), described in U.S. Application Serial No. 08/447,276), core-like antigen-adjacent proteins from the Human Immunodeficiency Virus (HIV) (such as those described in U.S. Application Serial No. 08/454,928), the envelope antigen of the Human T-cell Leukemia virus (HTLV) as described in U.S. Application Serial No. 08/454,928, the HBcAg antigen from HBV (*see, e.g.*, Principles and Practice of Clinical Virology, 3rd Edition, Chapter 2: Hepatitis Viruses, pp. 164-168), and the HPV 16 E7 antigen from the Human Papilloma Virus (HPV) (*see, e.g.*, Principles and Practice of Clinical Virology, 3rd Edition, Chapter 21: Papillomaviruses, pp. 621-623).

The determination of whether a given molecule is an antigen, and therefore a target antigen upon selection, is well within the skill of the art in view of the present specification. For example, whether a given molecule is antigenic can be determined by administering the molecule to an animal such as a rabbit or mouse, then observing whether the molecule induces an immune response.

"Serum albumin" is used herein in its traditional sense, to indicate a well known protein found in the blood. Serum albumins are secreted into the blood by liver cells, and bind to and solubilize many small molecules that are only slightly soluble in the blood serum absent such binding by the serum albumin. The folding of the polypeptide chain of serum albumin allows disulfide linkages to form between cysteine residues. The protein contains 3 similar protein domains, and is encoded by a gene having 14 introns and 15 exons. Thus, expression of the gene in eukaryotes and prokaryotes generally includes processing mechanisms or is performed using non-intron containing genes, such as cDNA. Darnell et al., *Molecular Cellular Biology*, Sci. Am. Books, 1986, pp. 413-415; Rothschild et al., *The New England Journal of Medicine 286*(14):748-757, 1972; Sjobring et al., *The Journal of Biological Chemistry 266*(1):399-405, 1991; Tullis, J., *J.A.M.A. 237*(4):355-360, 1977; Tullis, J., *J.A.M.A. 237*(5):460-463, 1977. In preferred embodiments, the serum albumin is human serum albumin, α-fetoprotein, bovine serum albumin, fetal bovine serum albumin, new born bovine serum albumin or mouse serum albumin. Also preferably, the serum albumin is selected from the same species as the sample source or recipient of the target antigen-albumin complex. However, particularly for *in vitro* uses such as most diagnostic and

detection assays, the albumin can be from another species. The serum albumin can be recombinant, purified from an animal source, or produced synthetically.

"Complex," or aggregate, is also used herein in its traditional sense, to indicate a dimer or multimer formed between one or more target antigens and one or more serum albumins. (*See*, *e.g.*, WO 95/33053.) The complex is believed to be formed by protein-protein interactions between the target antigen and the serum albumin, as opposed, for example, to fusion of ends of the proteins to one another (as through genetic engineering) or covalent cross-linking by intermediate agents such as glutaraldehyde. Complexes of the present invention can include larger multimers, and can have molecular weights in excess of 800kD. Although not required to make or use the present invention, it is believed that the complex may be presented in a micelle form. Upon formation of the complex, it is believed that the conformation of the target antigen is altered. Thus, the presentation of the target antigen in the body, in the presence of serum albumin, is different from the presentation that is found *in vitro* in the absence of serum albumin.

In one embodiment, the present invention provides a complex as described above wherein the complex comprises at least three components: a target antigen, a serum albumin, and a second target antigen that is different from the first target antigen. The second target antigen can provide a different antigenic site specific to a second pathogenic state, so that the immuno-inducing composition or assay is effective for more than the pathogenic state associated with the first target antigen. Surprisingly, the second target antigen can also provide an even further enhanced immunological response against the first target antigen (and vice-versa), and can similarly provide for an even further enhanced ability to detect the first target antigen in a sample. In another embodiment relating to multiple antigen-serum albumin complexes, one or more of the target antigens can be a core-like antigen-adjacent protein, such as those described in U.S. Application Serial No. 08/454,928. Such antigens provide not only enhanced effects in combination with the serum albumin, but also provide enhanced effects in combination with the other target antigen(s). In

preferred embodiments, the multiple antigens are selected from two or more of HCV, HIV and HBV, particularly the HBcAg antigen.

With respect to proteinacious target antigens, serum albumins and other proteins of the present invention, as discussed further below, it is within the skill of the art to make conservative amino acid substitutions, or insignificant amino acid additions, modifications, truncations or deletions, that may change the amino acid sequence of the protein but do not significantly alter the functioning of the protein (*e.g.*, the antigenic configuration is retained).

The present invention also provides antibodies, including monoclonal antibodies, to the conformationally changed target antigens and serum albumins of the present invention, as well as other proteins of the present invention. The antibodies can be used, for example, to provide particularly sensitive and specific detection of the target antigen in a sample.

In one embodiment, the present invention provides the isolation of serum albumin from a sample by the use of an antibody against the serum albumin. Serum albumin is deemed to be "isolated" from a sample within the context of the present invention if no other (undesired) protein is detected pursuant to SDS-PAGE analysis followed by coomassie blue staining. Within other embodiments, the serum albumin can be isolated such that no other (undesired) protein is detected pursuant to SDS-PAGE analysis followed by silver staining. Within still other embodiments, the serum albumin is isolated if no other protein having significant antigenic activity that significantly interferes with detection assays or immunological events is included with the serum albumin. Further, the isolation of a protein, including serum albumin from an animal means that the protein has been removed from the animal.

Still further, the present invention provides compositions and methods for the elicitation of an enhanced immune response in an animal (either humoral, cellular, or both). Even further, the compositions and methods can vaccinate an animal against the pathogenic state, such as an infectious agent associated with the target antigen.

In another aspect, the present invention provides the use of a serum albumin complexed with a drug to provide a drug-serum albumin complex. The term drug is used herein in its traditional meaning and need not be a protein or antigen. The complexing of the serum albumin with the drug enhances the effectiveness of the drug by at least about 1.1 times, and preferably at least about 1.25, 1.5 or 2.0 times when compared to the effectiveness of the drug alone. Typically, the drug-serum albumin complex is made and used in accordance with the uses of a target antigen-serum albumin complex discussed herein.

Preferably, the methods and compositions of the present invention, including those for detection, immune response elicitation and vaccination, are applied to a human being or to samples obtained from a human being. The sample can be obtained from blood, urine, sputum, feces, lymph or other traditional sources.

Protein Production

As noted above, the target antigens and serum albumins of the present invention can be either recombinant or purified from an animal source. Purification can be effected using methods well known in the art, including affinity chromatography, ion exchange ultrafiltration and gel filtration (The National Medical Series For Independent Study, "Chapter 3: Amino Acid and Protein Structure. IV. PURIFICATION OF PROTEINS," *Biochemistry*, 3d ed., pp. 37-42).

Production of the proteins of the present invention can also be effected using recombinant methods, which are generally well known in the art. Within such methods, numerous vector constructs suitable for use with a nucleic acid molecule encoding a desired protein can be prepared as a matter of convenience. Within the context of the present invention, a vector construct is understood to typically refer to a DNA molecule, or a clone of such a molecule (either single-stranded or double-stranded), that has been modified through human intervention to contain segments of DNA combined and juxtaposed in a manner that as a whole would not otherwise exist in nature. Vector constructs of the present invention comprise a first DNA segment encoding a desired protein operably linked to additional DNA segments required for the expression of the first DNA segment. Within the context of the present invention,

additional DNA segments will include a promoter and will generally include transcription terminators, and may further include operators, enhancers, mRNA ribosomal binding sites, appropriate sequences that control transcription and translation initiation and termination, and/or other elements. *See* WO 94/25597; WO 94/25598; Pouwels et al., *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985. The ability to replicate in the desired host cells, usually conferred by an origin of replication, and a selection gene by which transformants are identified, may additionally be incorporated into the vector construct.

Methods for producing recombinant target antigen or serum albumin in a variety of prokaryotic and eukaryotic host cells are generally known in the art (*Methods in Enzymology 185*, Goeddel (ed.), Academic Press, San Diego, Calif., 1990; *see also Methods in Enzymology*, Guthrie and Fink (eds.), Academic Press, San Diego, Calif., 1991), and are discussed in more detail below.

Turning to expression in bacterial hosts, vectors used for such expression will generally contain a selectable marker, such as a gene for antibiotic resistance, and a promoter that functions in the host cell. The expression units may also include a transcriptional terminator. Appropriate promoters include the following promoter systems: *trp* (Nichols and Yanofsky, *Meth. Enzymol. 101*:155-164, 1983), *lac* (Casadaban et al., *J. Bacteriol. 143*:971-980, 1980; Chang et al., *Nature 275*:615, 1978; Goeddel et al., *Nature 281*:544, 1979), phage λ (Queen, *J. Mol. Appl. Genet. 2*:1-10, 1983), β-lactamase (penicillinase); tryptophan (*trp*) (Goeddel et al., *Nucl. Acids Res. 8*:4057, 1980; and EP-A-36776), *tac* (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). Additionally, the phage λ P_L promoter can advantageously combined with a cI857ts thermolabile repressor sequence.

Examples of plasmids useful for transforming prokaryotic hosts include the pUC plasmids (Messing, *Meth. Enzymol. 101*:20-78, 1983; Vieira and Messing, *Gene 19*:259-268, 1982), pBR322 (Bolivar et al., *Gene 2*:95-113, 1977), pCQV2 (Queen, *ibid.*), pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), pGEM1 (Promega Biotec, Madison, WI, USA), pHUB2 (resident in *E. coli* strain JMB9 (ATCC

37092)), pPLc28 (resident in *E. coli* RR1 (ATCC 53082)), and derivatives thereof. Plasmids may contain viral, bacterial and/or eukaryotic elements.

Various prokaryotic host cells may be utilized within the context of the present invention. Generally, preferred prokaryotic host cells should have a well-characterized genetic system, including known cloning vectors and methods of genetic manipulation. Representative examples of such host cells include gram negative or gram positive organisms, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Enterobacteriaceae*, *Streptomyces*, *Staphylococcus*, *Bacillaceae*, *Salmonella*, *Shigella*, and *Corynebacteria*. In one preferred embodiment, the host cell is an *E. coli*, which can contain a DE3 lysogen and T7 RNA polymerase, such as BL21(DE3), JM109(DE3) or BL21(DE3) pLysS. In a prokaryotic host cell such as *E. coli*, a desired protein may include an N-terminal Methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant desired protein.

Desirable prokaryotes (and eukaryotes such as those discussed herein) may be readily obtained from a variety of commercial sources including, for example, the American Type Culture Collection (ATCC) (Rockville, Maryland). Alternatively, many of the above-described bacteria may be isolated from sources that are known by those of skill in the art to contain such prokaryotes, based upon techniques that are known in the art. (*See* Bergy's *Shorter Manual of Determinative Bacteriology*, 8th ed., Holt, John G. (ed.), Williams & Wilkins, 1977.)

Recombinant protein produced in prokaryotic culture is usually isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion chromatography steps. Finally, RP-HPLC can be employed for final purification steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Turning to production of desired protein in eukaryotic cells, eukaryotic host cells suitable for use in practicing the present invention include mammalian, avian, plant, insect and fungal cells such as yeast.

Yeast vectors will often contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. In addition, sequences encoding appropriate signal peptides that are not naturally associated with the desired protein can be incorporated into expression vectors. For example, a DNA sequence for a signal peptide (secretory leader) may be fused in-frame to the sequence encoding the desired protein so that the desired protein is initially translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells enhances extracellular secretion of the desired protein. The signal peptide may be cleaved from the desired protein upon secretion of the desired protein from the cell.

Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg. 7*:149, 1968; and Holland et al., *Biochem. 17*:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657 or in Fleer et al., *Gene 107*:285-295, 1991; and van den Berg et al., *Bio/Technology 8*:135-139, 1990. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem. 258*:2674, 1982) and Beier et al. (*Nature 300*:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α-factor leader sequence may be employed to direct secretion of an target antigen. The α-factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. *See, e.g.*, Kurjan et al., *Cell 30*:933, 1982; Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984; U.S. Patent 4,546,082; and EP 324,274. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA 75*:1929, 1978. The Hinnen et al. protocol selects for Trp^+ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil.

Yeast host cells transformed by vectors containing ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or insect host cell culture systems can also be employed to express desired recombinant proteins. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology 6*:47, 1988. Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell 23*:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV-1/EBNA-1 cell line derived from the African green monkey kidney cell line CVI (ATCC CCL 70) as described by McMahan et al. (*EMBO J. 10*:2821, 1991).

Transcriptional and translational control sequences for mammalian host cell expression vectors may be excised from viral genomes. Commonly used promoter

sequences and enhancer sequences are derived from Polyoma virus, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment which may also contain a viral origin of replication (Fiers et al., *Nature 273*:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Exemplary expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol. 3*:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol. 23*:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature 312*:768, 1984 has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in U.S. Patent Application Serial No. 07/701,415, filed May 16, 1991. The vectors may be derived from retroviruses. In place of the native signal sequence, a heterologous signal sequence may be added, such as the signal sequence for IL-7 described in United States Patent 4,965,195; the signal sequence for IL-2 receptor described in Cosman et al., *Nature 312*:768, 1984; the IL-4 signal peptide described in EP 367,566; the type I IL-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II IL-1 receptor signal peptide described in EP 460,846.

Preferred eukaryotic cells include cultured mammalian cell lines (*e.g.*, rodent or human cell lines), insect cell lines (*e.g.*, Sf-9) and fungal cells, including species of yeast (*e.g.*, *Saccharomyces* spp., particularly *S. cerevisiae*, *Schizosaccharomyces* spp., *Kluyveromyces* spp., *Pichia*, and *K. lactis*) or filamentous fungi (*e.g.*, *Aspergillus* spp., *Neurospora* spp.).

Techniques for transforming these host cells, and methods of expressing foreign DNA sequences cloned therein, are well known in the art (*see, e.g.*, Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1982; Sambrook et al., *supra*; "Gene Expression Technology," *Methods in Enzymology 185*, Goeddel (ed.), Academic Press, San Diego, Calif., 1990; "Guide to Yeast Genetics and Molecular Biology," *Methods in Enzymology*, Guthrie and Fink (eds.), Academic Press, San Diego, Calif., 1991; Hitzeman et al., *J. Biol. Chem. 255*:12073-12080, 1980; Alber and Kawasaki, *J. Mol. Appl. Genet. 1*:419-434, 1982; Young et al., in *Genetic Engineering of Microorganisms for Chemicals*, Hollaender et al. (eds.), Plenum, New York, 1982, p. 355; Ammerer, *Meth. Enzymol. 101*:192-201, 1983; McKnight et al., U.S. Patent No. 4,935,349).

Transformed yeast host cells are often preferable to mammalian or other eukaryotic systems to express desired protein as a secreted polypeptide in order to simplify purification. Secreted recombinant polypeptide from a yeast host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog. 296*:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

Turning to the sequences of the proteins of the present invention, including serum albumins and target antigens, and their encoding nucleic acid molecules, mutations in nucleotide sequences constructed for expression of the inventive proteins preferably preserve the reading frame of the encoding sequences. Furthermore, the mutations preferably do not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, that would adversely affect translation of the mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed mutants screened for indicative biological activity.

Mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling

ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a derivative having the desired amino acid insertion, substitution or deletion.

Alternatively, oligonucleotide-directed, site-specific mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene 42*:133, 1986); Bauer et al. (*Gene 37*:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and Sambrook et al. (*supra*).

The primary amino acid structure of the above-described proteins can also be modified by forming conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups, or with other proteins or polypeptides, provided that such modifications do not disrupt the antigenicity and/or cooperative properties of the proteins. (*See* U.S. Patent No. 4,851,341; *see also* Hopp et al., *Bio/Technology 6*:1204, 1988.) For example, such modifications should not interfere with the epitopic configuration (including access to the epitope and other antigenic considerations) that is specific to the target antigen when it is complexed with the serum albumin.

Cell-free translation systems can also be employed to produce desired proteins using RNAs derived from DNA constructs disclosed herein.

Antibodies

The present invention also provides antibodies, *i.e.*, binding partners, such as monoclonal and polyclonal antibodies, directed against the target antigens and serum albumins of the present invention. In a preferred embodiment, the antibodies comprise IgM and/or IgG antibodies, further preferably IgM antibodies. In a particularly preferred embodiment, the antibodies are produced using a target antigen that has been complexed with a serum albumin to provide a complex as one or both of the immunizing agent or the selective agent. As discussed above, such complexing is believed to induce conformational changes within the target antigen, so the specificity

and affinity of the antibodies are tailored to the target antigen as it is found within the body of an animal, thereby providing enhanced specificity and affinity when compared to that obtained using the target antigen when not complexed with a serum albumin.

Antibodies, including all forms of antibodies such as IgG and IgM antibodies, can be produced by using a protein/aggregate of the invention as an immunogen through standard procedures for preparing a hybridoma, and/or via other methods. The resulting antibodies are particularly useful for detecting the target antigen in a sample, preferably a sample from a human being. *See* WO 94/25597 and WO 94/25598.

Polyclonal antibodies can be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, turkeys, rabbits, mice, or rats. Briefly, the desired protein or peptide is utilized to immunize the animal, typically through intraperitoneal, intramuscular, intraocular, or subcutaneous injections. The immunogenicity of the protein or peptide of interest may be increased through the use of an adjuvant such as Freund's complete or incomplete adjuvant. Following several booster immunizations, small samples of serum are collected and tested for reactivity to the desired protein or peptide.

Once the titer of the animal has reached a plateau in terms of its reactivity to the protein, larger quantities of polyclonal antisera may be readily obtained either by weekly bleedings, or by exsanguinating the animal.

Monoclonal antibodies can also be readily generated using well-known techniques (*see* U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; *see also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennett et al. (eds.), Plenum Press, 1980, and *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Briefly, in one embodiment, a subject animal such as a rat or mouse is injected with a desired protein or peptide. If desired, various techniques may be utilized in order to increase the resultant immune response generated by the protein, in order to develop greater antibody reactivity. For example, the desired protein or peptide may be coupled to another protein such as ovalbumin or keyhole limpet hemocyanin (KLH), or through the

use of adjuvants such as Freund's complete or incomplete adjuvants. The initial elicitation of an immune response may be through intraperitoneal, intramuscular, intraocular, or subcutaneous routes.

Between one and three weeks after the initial immunization, the animal may be reimmunized with booster immunization. The animal may then be test bled and the serum tested for binding to the unprocessed polypeptide using assays as described above. Additional immunizations may also be accomplished until the animal has reached a plateau in its reactivity to the desired protein or peptide. The animal may then be given a final boost of the desired protein or peptide, and three to four days later sacrificed. At this time, the spleen and lymph nodes may be harvested and disrupted into a single cell suspension by passing the organs through a mesh screen or by rupturing the spleen or lymph node membranes which encapsulate the cells. Within one embodiment the red cells are subsequently lysed by the addition of a hypotonic solution, followed by immediate return to isotonicity.

Within another embodiment, suitable cells for preparing monoclonal antibodies are obtained through the use of *in vitro* immunization techniques. Briefly, an animal is sacrificed, and the spleen and lymph node cells are removed as described above. A single cell suspension is prepared, and the cells are placed into a culture containing a form of the protein or peptide of interest that is suitable for generating an immune response as described above. Subsequently, the lymphocytes are harvested and fused as described below.

Cells that are obtained through the use of *in vitro* immunization or from an immunized animal as described above may be immortalized by transfection with a virus such as the Epstein-Barr Virus (EBV). (*See* Glasky and Reading, *Hybridoma* *8*(4):377-389, 1989.) Alternatively, within a preferred embodiment, the harvested spleen and/or lymph node cell suspensions are fused with a suitable myeloma cell in order to create a "hybridoma" which secretes monoclonal antibodies. Suitable myeloma lines are preferably defective in the construction or expression of antibodies, and are additionally syngeneic with the cells from the immunized animal. Many such myeloma cell lines are well known in the art and may be obtained from sources such as the

American Type Culture Collection (ATCC), Rockville, Maryland (*see Catalogue of Cell Lines & Hybridomas*, 6th ed., ATCC, 1988). Representative myeloma lines include: for humans, UC 729-6 (ATCC No. CRL 8061), MC/CAR-Z2 (ATCC No. CRL 8147), and SKO-007 (ATCC No. CRL 8033); for mice, SP2/0-Ag14 (ATCC No. CRL 1581), and P3X63Ag8 (ATCC No. TIB 9); and for rats, Y3-Ag1.2.3 (ATCC No. CRL 1631), and YB2/0 (ATCC No. CRL 1662). Preferred fusion lines include NS-1 (ATCC No. TIB 18) and P3X63-Ag 8.653 (ATCC No. CRL 1580), which may be utilized for fusions with either mouse, rat, or human cell lines. Fusion between the myeloma cell line and the cells from the immunized animal can be accomplished by a variety of methods, including the use of polyethylene glycol (PEG) (*see Antibodies: A Laboratory Manual, supra*) or electrofusion (*see* Zimmerman and Vienken, *J. Membrane Biol. 67*:165-182, 1982).

Following the fusion, the cells are placed into culture plates containing a suitable medium, such as RPMI 1640 or DMEM (Dulbecco's Modified Eagles Medium, JRH Biosciences, Lenexa, Kan.). The medium may also contain additional ingredients, such as fetal bovine serum (FBS, *e.g.*, from Hyclone, Logan, Utah, or JRH Biosciences), thymocytes that were harvested from a baby animal of the same species as was used for immunization, or agar to solidify the medium. Additionally, the medium should contain a reagent which selectively allows for the growth of fused spleen and myeloma cells. Particularly preferred is the use of HAT medium (hypoxanthine, aminopterin, and thymidine) (Sigma Chemical Co., St. Louis, Mo.). After about seven days, the resulting fused cells or hybridomas may be screened in order to determine the presence of antibodies which recognizes the proteins of the present invention. Following several clonal dilutions and reassays, a hybridoma producing antibodies that bind to the protein of interest can be isolated.

Other techniques can also be utilized to construct monoclonal antibodies. (*See* Huse et al., "Generation of a Large Combinational Library of the Immunoglobulin Repertoire in Phage Lambda," *Science 246*:1275-1281, 1989; Sastry et al., "Cloning of the Immunological Repertoire in *Escherichia coli* for Generation of Monoclonal Catalytic Antibodies: Construction of a Heavy Chain Variable Region-Specific cDNA

Library," *Proc. Natl. Acad. Sci. USA 86*:5728-5732, 1989; Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas," *Strategies in Molecular Biology 3*:1-9, 1990; these references describe a commercial system available from Stratacyte, La Jolla, California, which enables the production of antibodies through recombinant techniques.) Briefly, mRNA is isolated from a B cell population and utilized to create heavy and light chain immunoglobulin cDNA expression libraries in the λIMMUNOZAP(H) and λIMMUNOZAP(L) vectors. These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (*see* Huse et al., *supra*; Sastry et al., *supra*). Positive plaques can subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from *E. coli*.

Similarly, antibodies can also be constructed utilizing recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specifically binding antibody. The construction of these antibodies can be readily accomplished by one of ordinary skill in the art given the disclosure provided herein. (*See* Larrick et al., "Polymerase Chain Reaction Using Mixed Primers: Cloning of Human Monoclonal Antibody Variable Region Genes From Single Hybridoma Cells," *Biotechnology 7*:934-938, 1989; Riechmann et al., "Reshaping Human Antibodies for Therapy," *Nature 332*:323-327, 1988; Roberts et al., "Generation of an Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering," *Nature 328*:731-734, 1987; Verhoeyen et al., "Reshaping Human Antibodies: Grafting an Antilysozyme Activity," *Science 239*:1534-1536, 1988; Chaudhary et al., "A Recombinant Immunotoxin Consisting of Two Antibody Variable Domains Fused to *Pseudomonas* Exotoxin," *Nature 339*:394-397, 1989; *see also* U.S. Patent No. 5,132,405 entitled "Biosynthetic Antibody Binding Sites.") Briefly, in one embodiment, DNA segments encoding the desired protein or peptide interest-specific antigen binding domains are amplified from hybridomas that produce a specifically binding monoclonal antibody, and are inserted directly into the genome of a cell that produces human antibodies. (*See* Verhoeyen et al., *supra*; *see also* Reichmann et al., *supra*.) This technique allows the antigen-binding site of a specifically binding mouse or rat monoclonal antibody to be

transferred into a human antibody. Such antibodies are preferable for therapeutic use in humans because they are not as antigenic as rat or mouse antibodies.

Monoclonal antibodies and other antibodies can be produced in a number of host systems, including tissue cultures, bacteria, eukaryotic cells, plants and other host systems known in the art.

Once suitable antibodies have been obtained, they may be isolated or purified by many techniques well known to those of ordinary skill in the art (*see Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; U.S. Patent No. 4,736,110; and U.S. Patent No. 4,486,530). Suitable isolation techniques include peptide or protein affinity columns, HPLC or RPHPLC, purification on protein A or protein G columns, or any combination of these techniques. Within the context of the present invention, the term "isolated" as used to define antibodies means substantially free of other blood components.

The antibodies of the present invention have many uses. As discussed further below, the antibodies of the present invention are particularly useful for the detection and diagnosis of target antigens and the pathogenic agents associated therewith. Other uses include, for example, flow cytometry to sort cells displaying one more of the target antigens of the present invention. Briefly, in order to detect the target antigens of interest on cells, the cells are incubated with a labeled monoclonal antibody which specifically binds to the protein of interest, followed by detection of the presence of bound antibody. These steps may also be accomplished with additional steps such as washings to remove unbound antibody. Labels suitable for use within the present invention are well known in the art including, among others, flourescein isothiocyanate (FITC), phycoerythrin (PE), horse radish peroxidase (HRP), and colloidal gold. Particularly preferred for use in flow cytometry is FITC, which may be conjugated to a purified antibody according to the method of Keltkamp in "Conjugation of Fluorescein Isothiocyanate to Antibodies. I. Experiments on the Conditions of Conjugation," *Immunology 18*:865-873, 1970. (*See also* Keltkamp, "Conjugation of Fluorescein Isothiocyanate to Antibodies. II. A Reproducible Method," *Immunology 18*:875-881,

1970; Goding, "Conjugation of Antibodies with Fluorochromes: Modification to the Standard Methods," *J. Immunol. Methods 13*:215-226, 1970.)

Assays For The Detection Of A Target Antigen in A Sample

As noted above, the present invention provides methods for detecting a target antigen in a sample. The methods, or assays, are typically based on the detection of antigens associated with a pathogenic agent, such as the antigens displayed by a positive-stranded RNA virus. The present invention also provides methods of detecting antibodies produced against a target antigen. The methods are characterized by the ability of the target antigen-serum albumin complex of the present invention to be bound by antibodies against the target antigen, and the ability of antibodies produced against the target antigen-serum albumin complex of the present invention to bind to target antigens in a sample. As noted above, these aspects of the invention preferably include the use of IgM and/or IgG antibodies.

Surprisingly, the target antigen-serum albumin complex, or antibodies produced against the complex, of the present invention provide significantly enhanced detection of target antigen. For example, with reference to HCV, the use of both an core-env target antigen complexed with a serum albumin in the assay provides a synergistic effect that permits significantly more sensitive detection of the target antigen than when either the core-env antigen or the serum albumin is utilized alone (indeed, the serum albumin alone provides essentially no detection of the target antigen or antibodies thereto).

A preferred assay for the detection of the target antigen is a sandwich assay such as an enzyme-linked immunosorbent assay (ELISA). In one preferred embodiment, the ELISA comprises the following steps: (1) coating target antigen complexed with at least one serum albumin onto a solid phase, (2) incubating a sample suspected of containing antibodies against the target antigen with the complex coated onto the solid phase under conditions that allow the formation of an antigen-antibody complex, (3) adding an anti-antibody (such as anti-IgG) conjugated with a label to be captured by the resulting antigen-antibody complex bound to the solid phase, and

(4) measuring the captured label and determining therefrom whether the sample has antibodies against the target antigen.

Although a preferred assay is set forth above, a variety of assays can be utilized in order to detect antibodies that specifically bind to a target antigen in a sample, or to detect a target antigen bound to one or more antibodies from the sample. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: countercurrent immuno-electrophoresis (CIEP), radioimmunoassays, radioimmunoprecipitations, enzyme-linked immunosorbent assays (ELISA), dot blot assays, inhibition or competition assays, sandwich assays, immunostick (dip-stick) assays, simultaneous assays, immunochromatographic assays, immunofiltration assays, latex bead agglutination assays, immunofluorescent assays, biosensor assays, and low-light detection assays (*see* U.S. Patent Nos. 4,376,110 and 4,486,530; WO 94/25597; WO/25598; *see also Antibodies: A Laboratory Manual*, *supra*).

A fluorescent antibody test (FA-test) uses a fluorescently labeled antibody able to bind to one of the target antigens of the invention. For detection, visual determinations are made by a technician using fluorescence microscopy, yielding a qualitative result. In one embodiment, this assay is used for the examination of tissue samples or histological sections.

In latex bead agglutination assays, antibodies made using the target antigen-serum albumin complex of the present invention are conjugated to latex beads. The antibodies conjugated to the latex beads are then contacted with a sample under conditions permitting the antibodies to bind to target antigens in the sample, if any. The results are then read visually, yielding a qualitative result. In one embodiment, this format can be used in the field for on-site testing.

Enzyme immunoassays (EIA) include a number of different assays able to utilize the antibodies provided by the present invention. For example, a heterogeneous indirect EIA uses a solid phase coupled with an antibody of the invention and an affinity purified, anti-IgG immunoglobulin preparation. Preferably, the solid

phase is a polystyrene microtiter plate. The antibodies and immunoglobulin preparation are then contacted with the sample under conditions permitting antibody binding, which conditions are well known in the art. The results of such an assay can be read visually, but are preferably read using a spectrophotometer, such as an ELISA plate reader, to yield a quantitative result. An alternative solid phase EIA format includes plastic-coated ferrous metal beads able to be moved during the procedures of the assay by means of a magnet. Yet another alternative is a low-light detection immunoassay format. In this highly sensitive format, the light emission produced by appropriately labeled bound antibodies are quantitated automatically. Preferably, the reaction is performed using microtiter plates.

In an alternative embodiment, a radioactive tracer is substituted for the enzyme mediated detection in an EIA to produce a radioimmunoassay (RIA).

In a capture-antibody sandwich enzyme assay, the target antigen is bound between an antibody attached to a solid phase, preferably a polystyrene microtiter plate, and a labeled antibody. Preferably, the results are measured using a spectrophotometer, such as an ELISA plate reader. This assay is one preferred embodiment for the present invention.

In a sequential assay format, reagents are allowed to incubate with the capture antibody in a step-wise fashion. The test sample is first incubated with the capture antibody. Following a wash step, an incubation with the labeled antibody occurs. In a simultaneous assay, the two incubation periods described in the sequential assay are combined. This eliminates one incubation period plus a wash step.

A dipstick/immunostick format is essentially an immunoassay except that the solid phase, instead of being a polystyrene microtiter plate, is a polystyrene paddle or dipstick. Reagents are the same and the format can either be simultaneous or sequential.

In a chromatographic strip test format, a capture antibody and a labeled antibody are dried onto a chromatographic strip, which is typically nitrocellulose or nylon of high porosity bonded to cellulose acetate. The capture antibody is usually spray dried as a line at one end of the strip. At this end there is an absorbent material

that is in contact with the strip. At the other end of the strip the labeled antibody is deposited in a manner that prevents it from being absorbed into the membrane. Usually, the label attached to the antibody is a latex bead or colloidal gold. The assay may be initiated by applying the sample immediately in front of the labeled antibody.

Immunofiltration/immunoconcentration formats combine a large solid phase surface with directional flow of sample/reagents, which concentrates and accelerates the binding of antigen to antibody. In a preferred format, the test sample is preincubated with a labeled antibody then applied to a solid phase such as fiber filters or nitrocellulose membranes or the like. The solid phase can also be precoated with latex or glass beads coated with capture antibody. Detection of analyte is the same as standard immunoassay. The flow of sample/reagents can be modulated by either vacuum or the wicking action of an underlying absorbent material.

A threshold biosensor assay is a sensitive, instrumented assay amenable to screening large numbers of samples at low cost. In one embodiment, such an assay comprises the use of light addressable potentiometric sensors wherein the reaction involves the detection of a pH change due to binding of the desired protein by capture antibodies, bridging antibodies and urease-conjugated antibodies. Upon binding, a pH change is effected that is measurable by translation into electrical potential (μvolts). The assay typically occurs in a very small reaction volume, and is very sensitive. Moreover, the reported detection limit of the assay is 1,000 molecules of urease per minute.

Compositions And Methods For The Elicitation Of An Immune Response Against A Target Antigen

The present invention also provides compositions and methods for the elicitation of an immune response to the target antigen-serum albumin complex, which response may be either humoral, cellular, or both. Preferably, the immune response is induced by a vaccine against the pathogenic agent associated with a target antigen, such as a positive-stranded RNA virus associated with its core/gag protein, and is therefore an immunoprotective immune response. These compositions and methods typically involve an immunogen comprising a target antigen-serum albumin complex of the

present invention in combination with a pharmaceutically acceptable carrier or diluent. The compositions can also include a cocktail of differing target antigens so that multiple disease states can be affected, or so that multiple aspects of a single disease state can be affected. Such a cocktail can include a variety of target antigens, with each complexed with its own serum albumin, or the cocktail can include a plurality of target antigens aggregated with the serum albumin in a single complex. The target antigen may comprise an inactivated preparation or an attenuated preparation.

Accordingly, another aspect of the present invention provides target antigen-serum albumin complexes capable of eliciting an immune response, preferably capable of immunizing an animal. As will be understood by one of ordinary skill in the art, with respect to the target antigens of the present invention, slight deviations of the amino acid sequences can be made without affecting the immunogenicity of the immunogen. Substantial equivalents of the target antigens include conservative substitutions of amino acids that maintain substantially the same charge and hydrophobicity as the original amino acid. Conservative substitutions include replacement of valine for isoleucine or leucine, and aspartic acid for glutamic acid, as well as other substitutions of a similar nature (*see* Dayhoff et al. (eds.), "Atlas of Protein Sequence and Structure," *Natl. Biomed. Res. Fdn.*, 1978).

As will be evident to one of ordinary skill in the art, the immunogens listed above, including their substantial equivalents, may stimulate different levels of response in different animals. The immunogens listed above, including their substantial equivalents, can be tested for effectiveness as a vaccine. These tests include T-cell proliferation assays, determination of lymphokine production after stimulation, and immunoprotection trials. Briefly, T-cell proliferation assays can be utilized as an indicator of potential for cell-mediated immunity. Additionally, evidence of lymphokine production after stimulation by an immunogen can be utilized to determine the potential for protection provided by an immunogen.

Finally, as described below, actual immunoprotection trials can be performed in order to determine protection in animals. In the case of humans, however, instead of immunoprotection trials it is preferred to first screen peripheral blood

lymphocytes (PBLs) from patients infected with pathogenic agents in the following manner. Briefly, PBLs can be isolated from diluted whole blood using Ficoll density gradient centrifugation and utilized in cell proliferation studies with [^{3}H]-thymidine as described below. Positive peptides are then selected and utilized in primate trials.

The immunogens, or polypeptides, of the present invention can be readily produced utilizing many other techniques well known in the art (*see* Sambrook et al., *supra*, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989).

Immunogens comprising the target antigen-serum albumin complexes of the present invention in combination with a pharmaceutically acceptable carrier or diluent can be administered to a patient in accordance with a number procedures known in the art. *See* WO 94/25597 and WO 94/25598.

For purposes of the present invention, warm-blooded animals include, among others, humans, primates, rabbits and mice, chickens and other fowl, cats and dogs, cattle and horses.

Many suitable carriers or diluents can be utilized in the present invention, including among others saline, buffered saline, and saline mixed with nonspecific serum albumin. The pharmaceutical composition may also contain other excipient ingredients, including adjuvants, buffers, antioxidants, carbohydrates such as glucose, sucrose, or dextrins, and chelating agents such as EDTA. Although the serum albumin itself acts as an adjuvant, thereby enhancing the immune response to the target antigen, other adjuvants may also be utilized along with the immunogen, if desired. Examples of such adjuvants include alum or aluminum hydroxide for humans.

The amount and frequency of administration can be determined in clinical trials, and may depend upon such factors as the nature of the target antigen, the pathogenic agent with which the target antigen is associated, the degree of protection required, and other considerations. In one embodiment, immunizations will involve oral administration. Alternatively, the vaccine can be parenterally administrated via the subcutaneous route, or via other routes. Depending upon the application, quantities of injected immunogen will vary from 50 μg to several milligrams in combination with a

pharmaceutically acceptable carrier or diluent, and will typically vary from about 100 μg to 1 mg. Booster immunizations can be given from 4-6 weeks later.

Kits For Implementation Of The Various Aspects Of The Claimed Invention

The present invention further provides kits for analyzing samples for the presence of target antigens or antibodies. The kits comprise a target antigen-serum albumin complex or antibody against the same and an appropriate solid phase. Preferably, the target antigen-serum albumin complex or antibody is bound to the solid phase. The kits can also provide one or more reagents and/or devices for the detection of the target antigen-serum albumin complex or antibodies. A variety of formats, reagents and devices for inclusion within the kits, including means for detecting the antigens or antibodies, are discussed herein.

The present invention also provides kits for the induction of an immune response. The kits comprise compositions comprising a target antigen-serum albumin complex of the invention in combination with an pharmaceutically acceptable carrier or diluent, and can also provide devices for administering or assisting in the administration of the composition.

Other kits suitable for use with the features of the present invention are also provided herewith.

The following Examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

ELISA FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HUMAN SERUM ALBUMIN

An ELISA was used to test samples from Hepatitis C Virus (HCV) positive and negative patients for the presence of antibodies against human serum albumin (HSA). The ELISA was performed as follows.

(A) Preparation Of Antigens

Human serum albumin (No. A-8763, Sigma, St. Louis, Missouri, USA) in a stock solution at 1 mg/ml in distilled water was added to coating buffer to provide a final concentration of 1.5 μg/ml, and mixed at room temperature for 60 minutes. 100 μl/well of this solution was coated onto a microtiter plate (Nunc-Immuno Strip, MaxiSorp, Denmark) and blocked with a buffer containing bovine serum albumin. The plate was incubated for 24 hours in a 40°C incubator. Next, the wells were washed, then postcoating buffer (0.01M NaH_2P0_4 H_2O/Na_2HPO_4 $7H_2O/0.15N$ NaCl/1% BSA, pH 7.2-7.4) was added into each of the wells. The plate was then let stand either overnight at 4°C or at room temperature for 3 hours.

(B) Preparation of Anti-hIgG:HRPO Conjugate

Purified anti-human Immunoglobulin G (anti-hIgG) was conjugated with horse radish peroxidase (HRPO) using $NaIO_4$ to obtain the anti-IgG:HRPO conjugate. The conjugate was purified by S-200 gel filtration and was diluted in sample diluent (0.1M Tris-HCl pH: 7.4±0.2, 40% NBBS, 1% BSA, 2% mouse serum).

(C) Components of Reagents

(a) coating buffers:

TNSCN coating buffer: 50 mM Tris-HCl/0.15 N NaCl/3 M NaSCN, pH: 7.4 ± 0.2.

TNEU coating buffer: 50 mM Tris-HCl/0.15 N NaCl/1 mM EDTA/6 M urea, pH 7.2-7.4.

Carbonate coating buffer: 0.1 M $NaHCO_3/Na_2CO_3$, pH 9.6±0.2.

PBS (phosphate buffered saline) coating buffer: 0.01 M Na phosphate buffer ($NaH_2PO_4 \cdot H_2O/Na_2HPO_4 \cdot 7H_2O$/0.15 N NaCl, pH 7.2-7.4).

TNSDS coating buffer: 50 mM Tris-HCl/0.15 N NaCl/0.02% SDS, pH 7.4±0.2.

(b) Wash Solution: Phosphate buffer, 0.01 M (KH_2PO_4/Na_2HPO_4)/0.15 N NaCl/0.05% Tween 20/0.001% thimerosol.

(c) Anti-hIgG:HRPO Conjugate Solution: as described above.

(d) Sample Diluent: 0.1M Tris-HCl pH: 7.4±0.2, 40% NBBS, 1% BSA, 2% mouse serum.

(e) OPD tablets, purchased from Beckman (Nyon, Switzerland): O-phenylene diamine (OPD) dissolved in citrate-phosphate buffer containing H_2O_2 (if the solution becomes orange, it means that the solution has been contaminated and cannot be used any more).

(f) Substrate diluent: Citrate-phosphate buffer (4.666 g Citric acid and 7.299 g Na_2HPO_4 dissolved in 1 liter distilled water) containing H_2O_2 (final concentration: 0.0125%).

(g) Substrate solution: 1 OPD tablet dissolved in 5 ml substrate diluent.

(h) Stopping Solution: 2N H_2SO_4.

(i) Positive/Negative controls: The serum samples of persons infected with/without hepatitis C diluted with sample diluent.

(D) Procedure:

(a) 150 µl sample diluent and 15 µl of sample, and positive/negative controls, were added into each well of the treated microtiter plate. Some wells were retained as substrate blanks.

(b) The plate was gently mixed by shaking and incubated at 40±1°C for 60±2 minutes.

(c) The plate was washed three times with 0.3 ml of Wash solution per well.

(d) 100 µl of anti-human IgG:HRPO Conjugate Solution was added to each well (except blank wells).

(e) The plate was gently mixed by shaking and incubated at 40±1°C for 30±2 minutes.

(f) The plate was washed six times using washing buffer.

(g) 100 µl of OPD Substrate Solution (1 OPD tablet dissolved in 5 ml diluent substrate) was added to each well and the plate was incubated at room temperature in the dark for 30 minutes.

(h) 100 µl of Stopping Solution was added to each well and gently mixed to stop the reaction.

(E) Determination:

The OD value per well was measured at 492 nm in a spectrophotometer. The OD_{492nm} value per well subtracts the mean of the readings of the blanks (backgrounds). The difference (PCx-NCx) between the mean of the readings of the positive controls (PCx) and that of the negative controls (NCx) is equal to or more than 0.5.

The cut-off value (CO) is calculated by the following formula:

$$CO = PCx \times 0.15 + NCx$$

When the readings from test samples were less than the CO value, the samples were considered negative (*i.e.*, anti-HSA antibodies could not be detected in the samples).

When the readings of test samples were equal to or more than the CO value, the samples were expected to be positive; however, it is preferred to repeat the assay for the samples in duplicate. If the readings of either of the duplicate samples were less than the CO value, the samples were considered to be negative. If the duplicate samples were both more than or equal to the Cut-off value, the samples were considered to be positive.

When the readings of test samples are more than NCx but less than the CO value by 20%, the samples should be regarded as questionable samples and the assay has to be repeated for those samples.

The samples for the experiment reported in Tables 1, 2 and 3 were as follows. S-2, S-3, S-4, S-6, 7-1, 7-2, 7-3, 7-4, 7-5, 7-6, 7-7, 7-8, 7-10, 7-11, 7-12, 7-13, 7-14, 7-15, 7-16, 7-17, 7-18, 7-19, and 7-20 were obtained from Blood donors. N753, N754, N755, N756, N757, N758, N759, N760, N761, N762, N763, N764, N765, N766, N767, N768, N769, N770, N771, N783, N784, N785, N786 and N787 were obtained from Patient sera.

Table 1

Samples	Anti-HCV	TNEU Anti-HSA	C.B. Anti-HSA	PBS Anti-HSA
7-1	+@	> 2.0 #	0.152	0.308
7-2	+	0.480	0.052	0.204
7-3	+	0.405	0.093	0.125
7-4	+	0.165	0.013	0.040
7-5	+	> 2.0	0.024	0.137
7-6	+	0.957	0.040	0.067
7-7	+	0.185	0.015	0.056
7-8	+	0.737	0.021	0.106

Samples	Anti-HCV	TNEU Anti-HSA	C.B. Anti-HSA	PBS Anti-HSA
7-10	+	0.278	0.031	0.065
7-11	+	0.788	0.036	0.118
7-12	+	0.825	0.046	0.088
7-13	+	0.711	0.034	0.144
7-14	+	> 2.0	0.079	0.144
7-15	+	0.118	0.006	0.038
7-16	+	1.903	0.037	0.145
7-17	+	> 2.0	0.059	0.325
7-18	+	0.922	0.023	0.093
7-19	+	> 2.0	0.074	0.203
7-20	+	0.760	0.029	0.159
N753	- $	0.017	0.023	0.022
N754	-	0.018	0.054	0.022
N755	-	0.044	0.039	0.047
N756	+	0.452	0.053	0.104
N757	+	0.271	0.045	0.099
N758	-	0.026	0.028	0.035
N759	-	0.021	0.021	0.036
N760	+	0.245	0.030	0.078
N761	+	0.597	0.055	0.143
N762	-	0.024	0.017	0.020
N763	-	0.022	0.023	0.023
N764	-	0.047	0.035	0.031
N765	+	0.163	0.018	0.044
N766	-	0.010	0.007	0.016
N767	-	0.007	0.012	0.017
N768	+	1.889	0.045	0.152

Samples	Anti-HCV	TNEU Anti-HSA	C.B. Anti-HSA	PBS Anti-HSA
N769	+	0.362	0.200	0.122
N770	+	0.652	0.053	0.133
N771	+	> 2.0	0.093	0.208

@: Reactive for anti-HCV.
#: Absorbance at 492 nm.
$: Non-reactive for anti-HCV.

Table 2

Samples	Anti-HCV	Anti-HSA (TNSCN)
N743	+ $	> 2.0 #
N748	+	0.889
N749	- @	0.001
N750	-	0.035
N760	+	1.614
N761	+	> 2.0
N762	-	0.019
N763	-	0.032
N764	-	0.012
N765	+	0.506
N766	-	0.014
N767	-	0.018
N768	+	> 2.0
N769	+	> 2.0
N770	+	> 2.0
N771	+	> 2.0
N783	+	> 2.0
N784	-	0.017
N785	+	> 2.0
N786	-	0.011

Samples	Anti-HCV	Anti-HSA (TNSCN)
N787	-	0.011

$: Reactive for anti-HCV.
#: Absorbance at 492nm.
@: Non-reactive for anti-HCV.

Table 3

Samples	Anti-HCV	Anti-HSA (TNSDS)
7-8	+ $	> 2.0 #
7-10	+	> 2.0
7-11	+	> 2.0
7-12	+	> 2.0
7-13	+	> 2.0
7-14	+	> 2.0
7-15	+	> 2.0
7-16	+	> 2.0
7-17	+	> 2.0
7-18	+	> 2.0
7-19	+	> 2.0
7-20	+	> 2.0
S-2	- @	0.026
S-3	-	0.021
S-4	-	0.011
S-6	-	0.022

@: Reactive for anti-HCV.
#: Absorbance at 492 nm.
$: Non-reactive for anti-HCV.

The data in Tables 1, 2 and 3 show that anti-HSA antibodies were detected in samples from human beings. The antibodies were primarily detected in anti-HCV positive samples, but were typically not detected in non-infected individuals.

EXAMPLE 2

ASSAYS USING AN HSA-HCV ANTIGEN COMPLEX FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HCV

First Assay

An ELISA was performed as set forth in Example 1, except that a core-env fusion protein of Hepatitis C Virus (HCV) (EN-80-2 antigen, U.S. Application Serial No. 08/447,276) was mixed with the HSA during the preparation of the antigens, in TNEU coating buffer, to provide a complex of the HSA and the HCV antigen.

The samples for the assay were as follows. Anti-HCV positive samples were N8, N12, N9, N81, N302, N141, N93, N210, N207, N117, N10, N71, N49, N451, N505, N103 and N26. Anti-HCV negative samples were N626, N639, N634, N632, N209, N202, N203, N997, N986 and N208.

Table 4

Samples	HSA	EN-80-2	HSA & EN-80-2
N8/25X @	0.006 #	0.967	1.497
/50X	0.006	0.623	0.995
/100X	0.010	0.238	0.414
Sample Diluent $	0.010	0.006	0.004
N12/25X	0.012	0.632	1.373
/50X	0.003	0.307	0.517
/100X	0.001	0.138	0.231
N626	0.026	0.025	0.028
N9/25X	0.012	0.298	0.453
/50X	0.005	0.082	0.134
/100X	0.000	0.037	0.093
N639	0.044	0.086	0.091

Samples	HSA	EN-80-2	HSA & EN-80-2
N81/336X	0.016	0.618	1.378
/672X	0.011	0.247	0.464
/1344X	0.001	0.125	0.202
N634	0.033	0.049	0.077
N302/336X	0.010	0.419	0.686
/672X	0.009	0.211	0.235
/1344	0.008	0.075	0.129
N632	0.027	0.025	0.032
N26/10X	0.015	0.824	1.629
/20X	0.006	0.299	0.472

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492 nm.
$: Sample diluent: 0.1 M Tris-HCl, pH: 7.4 ± 0.2 with 40% new born bovine serum, 1% BSA and 2% mouse serum.

Second Assay

An ELISA was performed as set forth in the first assay of this Example 2, except that recalcified human serum (HS; non-reactive for HBV, HCV, HIV and HTLV) was mixed with the core-envelope fusion protein of Hepatitis C Virus (HCV) to provide a complex of the serum albumin in the HS and the HCV antigen.

Table 5

Samples	HS	EN-80-2	HS & EN-80-2
N141/105X	0.131 #	0.215	0.939
N93/51X	0.147	0.191	0.690
N81/1323X	0.144	0.192	0.584
N210/105X	0.156	0.582	1.639
N207/441X	0.108	0.400	0.850
N117/51X	0.140	0.147	0.444
N10/10X	0.122	0.206	0.505

Samples	HS	EN-80-2	HS & EN-80-2
N209	0.114	0.042	0.152
N71/42X	0.113	0.243	0.430
N302/441X	0.154	0.399	1.367
EH &	0.121	0.062	0.146

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492 nm.
&: Recalcified human serum, non-reactive for HBV, HCV, HIV and HTLV.

Third Assay

An ELISA was performed as set forth in the first and second assays of this Example 2, except that bovine serum albumin (BSA; Sigma, St. Louis, Missouri, USA) was mixed with the core-env fusion protein of Hepatitis C Virus (HCV) to provide a complex of the BSA and the HCV antigen.

Table 6

Samples	BSA	EN-80-2	BSA & EN-80-2
N8/25X @	0.008 @	0.967	> 2.0
/50X	0.000	0.623	0.744
/100X	0.022	0.238	0.222
Sample Diluent	0.000	0.006	0.001
N12/25X	0.014	0.632	1.914
/50X	0.002	0.307	0.491
/100X	0.006	0.138	0.194
N626	0.014	0.025	0.020
N9/25X	0.002	0.298	0.604
/50X	0.006	0.082	0.152
/100X	0.000	0.037	0.050
N639	0.060	0.086	0.052
N81/336X	0.018	0.618	1.697

Samples	BSA	EN-80-2	BSA & EN-80-2
/672X	0.004	0.247	0.619
/1344X	0.007	0.125	0.212
N634	0.025	0.049	0.019
N302/336X	0.021	0.419	0.488
/672X	0.023	0.211	0.389
/1344	0.011	0.075	0.107
N632	0.018	0.025	0.023
N26/10X	0.022	0.824	1.288
/20X	0.019	0.299	0.477

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492 nm.

Fourth Assay

An ELISA was performed as set forth in the first and second assays of this Example 2, except that fetal bovine serum (FBS; CSL, Victoria, Australia) was mixed with the core-env fusion protein of Hepatitis C Virus (HCV) to provide a complex of the serum albumin in the FBS and the HCV antigen.

Table 7

Samples	FBS	EN-80-2	FBS & EN-80-2
N141/105X @	0.211	0.215	0.527
N93/51X	0.046	0.191	0.325
N81/1323X	0.040	0.192	0.427
N210/105X	0.065	0.582	1.315
N207/441X	0.038	0.400	0.752
N10/10X	0.041	0.206	0.535
N209	0.033	0.042	0.035
N302/441X	0.066	0.399	1.039

Samples	FBS	EN-80-2	FBS & EN-80-2
EH	0.070	0.062	0.118

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492 nm.
&: Recalcified human serum, non-reactive for HBV, HCV, HIV and HTLV.

Fifth Assay

An ELISA was performed as set forth in the first and second assays of this Example 2, except that a partial core antigen of HCV (EN-80-5, having a molecular weight about 15,000 daltons as measured by electrophoresis through an SDS-polyacrylamide gel; *see* U.S. Application Serial No. 08/454,928) was mixed with the HSA in carbonate buffer to provide a complex of the HSA and the HCV antigen.

Table 8

Samples	HSA	EN-80-5	HSA+EN-80-5
N49/9X @	0.041 #	> 2.0	> 2.0
/29X	0.017	0.535	0.833
/81X	0.010	0.107	0.176
N505/27X	0.037	> 2.0	> 2.0
/81X	0.025	0.580	0.864
/243X	0.017	0.136	0.180
N997	0.035	0.061	0.050

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492nm.

Sixth Assay

An assay was performed as set forth in the first assay of this Example 2, except that mouse serum albumin (MSA; Sigma, St. Louis, Missouri, USA) was mixed with the core-envelope fusion protein of Hepatitis C Virus (HCV) to provide a complex of the MSA and HCV antigen.

Table 9

Samples	MSA	EN-80-2	MSA+EN-80-2
N451/27X @	0.038 #	0.578	1.143
/81X	0.022	0.147	0.281
/243X	0.012	0.046	0.074
N505/27X	0.066	> 2.0	> 2.0
/81X	0.025	0.415	0.986
/243X	0.015	0.131	0.203
N986	0.032	0.042	0.037

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492nm.

Seventh Assay

An ELISA was performed as set forth in the first assay of this Example 2 using TNEU coating buffer, and as antigens, HSA, EN-I-6 (an *env* antigen of the Human Immunodeficiency virus-1 (HIV-1), discussed further below), and the core-env fusion protein of HCV, as set forth in Table 10.

Table 10

Samples	HSA	I-6	80-2	HSA+ 80-2	I-6+ 80-2	I-6+ 80-2+ HSA
N103/81X @	0.017 #	0.016	0.277	0.522	0.459	0.827
/162X	0.014	0.012	0.135	0.201	0.192	0.330
N505/81X	0.036	0.041	1.100	> 2.0	1.471	> 2.0
/162X	0.025	0.009	0.523	0.728	0.521	1.129
/324X	0.010	0.010	0.208	0.298	0.259	0.441
S.D. $	0.010	0.015	0.010	0.007	0.023	0.047

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492nm.
$: Sample diluent.

These data showed both a cooperative interactive effect between HSA and EN-80-2 and between I-6 and EN-80-2, as well as an enhanced cooperative effect between the three antigens HSA, EN-80-2 and EN-I-6. This indicates that the cooperative interactions between HSA and EN-80-2 may be different from the cooperative interactions between EN-I-6 and EN-80-2, and also that the cooperative interactions of the two-component complexes may be partly or wholly different from the cooperative interactions of the three-component complexes. Such enhanced interactions that are found in the three-component complexes may be additive, or may be actually working in concert with each other.

EXAMPLE 3

ASSAYS USING AN HSA-HIV-1 ANTIGEN COMPLEX FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HIV-1

First Assay

An ELISA was performed as set forth Example 1 above, except that an *env* antigen of the Human Immunodeficiency Virus-1 (HIV-1) (EN-I-6, a recombinant fusion protein comprising the amino-terminal fragment of β-galactosidase (311 a.a.) fused to amino acids 474-863 of *env*, *i.e.*, gp160, having 705 a.a. (including spacer amino acids) and a Mw of 80.7 kDa; *see* U.S. Application Serial No. 08/454,928) was mixed with the HSA in TNEU buffer to provide a complex of the HSA and the HIV-1 *env* antigen. The samples for the assay were as follows. Anti-HIV-1 positive samples were T1, T2, T3, T4, T5, T10 and T14. Anti-HIV-1 negative samples were N977, N966, N968, N764, N766 and N952.

Table 11

Sample	HSA	EN-I-6	HSA & EN-I-6
T1/24X @	0.020 #	0.381	0.632
/72X	0.021	0.239	0.337
/216X	0.018	0.120	0.151
N977	0.035	0.038	0.040
T2/24X	0.019	0.464	0.564
/72X	0.015	0.228	0.316
/216X	0.012	0.107	0.159
Sample Diluent	0.000	0.003	0.012
T3/4X	0.025	0.210	0.394
/8X	0.015	0.199	0.304
/16X	0.017	0.094	0.140
N966	0.023	0.025	0.022
T5/24X	0.016	0.227	0.381
/72X	0.015	0.184	0.255
/216X	0.018	0.075	0.093
N968	0.020	0.028	0.029

@: Anti-HIV-I positive samples diluted with sample diluent.
#: Absorbance at 492 nm.

Second Assay

An ELISA was performed as set forth in the first assays of this Example 3, except that a *gag* antigen of the Human Immunodeficiency Virus-1 (HIV-1) (EN-I-5, a recombinant fusion protein comprising the amino-terminal fragment of β-galactosidase (377 a.a.) fused to *gag* p17 (a.a. 15-132) followed by *gag* p24 (a.a. 133-363) followed by *gag* p15 (a.a. 364-437), having 831 a.a. (including spacer amino acids) and a Mw of 92.8 kDa; *see* U.S. Application Serial No. 08/454,928) was mixed with the HSA in TNSCN buffer to provide a complex of the HSA and the HIV-1 *gag* antigen.

Table 12

Samples	HSA	EN-I-5	HSA +EN-I-5
T1	0.026 #	1.101	1.471
T4	0.046	0.295	0.740
T5	0.072	0.262	0.357
T10	0.037	0.837	1.016
T14/24X @	0.004	0.606	1.066
/72X	0.002	0.314	0.487
/214X	0.004	0.111	0.158
N764	0.034	0.041	0.032
N766	0.035	0.074	0.058

#: Absorbance at 492nm.
@: Anti-HIV-1 positive sample diluted with sample diluent.

Third Assay

An ELISA was performed as set forth in the first assay of this Example 3, using TNEU as the coating buffer, and, as antigens, HSA, EN-I-5, EN-I-6, and EN-80-2 as set forth in Table 13.

Table 13

Samples	HSA	I-5	I-6	80-2	HSA+ I-6	I-5+ I-6	80-2+ I-6	I-6+ 80-2+ I-5	I-6+ 80-2+ HSA
T4/640X @	0.005	0.009	1.638	0.010	1.855	> 2.0	> 2.0	> 2.0	> 2.0
/1280X	0.006	0.010	0.781	0.012	0.882	1.027	1.148	1.208	1.265
T14/320X	0.006	0.026	1.029	0.009	1.184	1.320	1.452	1.751	1.685
/640X	0.004	0.017	0.456	0.014	0.516	0.568	0.588	0.691	0.634
/1280X	0.010	0.013	0.182	0.011	0.228	0.246	0.244	0.291	0.296
N952/20X	0.010	0.012	0.063	0.014	0.076	0.066	0.055	0.088	0.110
/40X	0.015	0.012	0.029	0.012	0.033	0.047	0.036	0.059	0.040
/80X	0.011	0.017	0.016	0.005	0.015	0.024	0.024	0.060	0.045

Samples	HSA	I-5	I-6	80-2	HSA+ I-6	I-5+ I-6	80-2+ I-6	I-6+ 80-2+ I-5	I-6+ 80-2+ HSA
S.D. $	0.025	0.017	0.027	0.019	0.046	0.042	0.041	0.048	0.047

@: Samples diluted with sample diluent.
#: Absorbance at 492nm.
$: Sample diluent.

These data showed cooperative interactive effects between each of double component complexes HSA and EN-I-6, EN-I-5 and EN-I-6, and EN-80-2 and I-6. In addition, these data show an enhanced cooperative effect between the triple-component complexes EN-I-6, EN-80-2 and EN-I-5, or EN-I-6, EN-80-2 and HSA. This indicates that the cooperative interactions between HSA and EN-I-6 may be different from the cooperative interactions between EN-I-5 and EN-I-6, or between EN-I-6 and EN-80-2, and also that the cooperative interactions of the two-component complexes may be partly or wholly different from the cooperative interactions of the three-component complexes. Such enhanced interactions that are found in the three-component complexes may be additive, or may be actually working in concert with each other. Thus, as noted previously, the present invention provides target antigen-serum albumin complexes further comprising a third antigen.

Fourth Assay

An ELISA was performed as set forth in the first assay of this Example 3, except that the EN-I-5 *gag* antigen of the Human Immunodeficiency Virus 1 (HIV-1) (EN-I-5) was mixed with the HSA in PBS to provide a complex of the HSA and the HIV-1 gag antigen.

Table 14

Samples	HSA	I-5	HSA+ I-5
T14 original	0.065 @	0.820	1.992
T14/20X #	0.011	0.092	0.406
T14/40X	0.003	0.067	0.119

Samples	HSA	I-5	HSA+ I-5
T14/80X	0.001	0.050	0.052
T14/160X	0.005	0.026	0.038
N952/20X	0.003	0.004	0.006
/40X	0.001	0.002	0.004
S.D. $	0.005	0.002	0.004

@: Absorbance at 492nm.
#: Samples diluted with sample diluent.
$: Sample diluent.

EXAMPLE 4

ASSAYS USING AN HSA-HTLV-1 ANTIGEN COMPLEX FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HTLV-1

An assay was performed as set forth in the Example 1, above, except that an H1 antigen from the HTLV-1 envelope region was mixed with the HSA in TNEU buffer to provide a complex of the HSA and the HTLV-1 *env* antigen. The samples for the assay were as follows. Anti-HTLV-1 positive samples were SP, BBI 23 and BBI 25. Anti-HTLV-1 negative samples were N966 and N968.

Table 15

Solid phase	HSA	H1	HSA + H1
SP 80X	0.000	0.412	0.504
40X	0.009	0.726	1.185
20X	0.012	1.362	> 2.0
N966	0.009	0.007	0.013
BBI 23/10X	0.037	1.209	1.448
/30X	0.049	0.253	0.331
/90X	0.027	0.057	0.083
BBI 25/10X	0.027	0.610	0.766
/20X	0.038	0.256	0.288

Solid phase	HSA	H1	HSA + H1
/40X	0.029	0.098	0.124
/80X	0.029	0.059	0.067
N968	0.020	0.023	0.034

@: Anti-HTLV positive samples diluted with sample diluent.
#: Absorbance at 492nm.

EXAMPLE 5

ASSAYS USING AN HSA-HBV ANTIGEN COMPLEX FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HBV

An assay was performed as set forth in the Example 1, above, except that an HBcAg antigen (identified as A5) from the Hepatitis B Virus (HBV) was mixed with the HSA in TNEU buffer to provide a complex of the HSA and the HBcAg antigen of HBV. The samples for the assay were as follows. Anti-HBc positive samples were SP and N639. Anti-HBc negative samples were NC1 and NC2.

Table 16

Solid Phase	HSA	A5	HSA & A5
SP 2X @	0.024	1.096	> 2.0
4X	0.011	0.897	1.086
8X	0.007	0.389	0.578
16X	0.011	0.333	0.548
32X	0.000	0.193	0.204
0X	0.017	0.005	0.010
NC 1	0.012	0.009	0.015
NC 2	0.026	0.018	0.031
N639	0.014	0.881	1.715
N639 2X	0.024	0.803	1.280

@: Anti-HBc positive samples diluted with recalcified human serum, non-reactive for HBV, HCV and HIV.
#: Absorbance at 492 nm.

EXAMPLE 6

ASSAYS USING AN HSA-HPV ANTIGEN COMPLEX FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HPV

An assay was performed as set forth in the Example 1, above, except that a human papillomavirus (HPV) type 16 E7 antigen, identified as EN-P-1 was mixed with the HSA in TNEU buffer to provide a complex of the HSA and the E7 antigen of HPV. The samples for the assay were as follows. Anti-HPV positive samples were B7, B9 and B12. Anti-HPV negative samples were N966, N968, N977 and B14.

Table 17

Solid phase	HSA	EN-P-1	HSA + EN-P-1
B7/4X @	0.009 #	1.292	1.490
/8X	0.005	0.414	0.568
/16X	0.005	0.161	0.176
N966	0.022	0.037	0.044
B9/64X	0.009	1.274	1.939
/128X	0.012	0.565	0.799
/256X	0.014	0.228	0.298
/512X	0.015	0.102	0.141
B12/8X	0.005	1.544	1.725
/16X	0.003	0.585	0.693
/32X	0.000	0.244	0.286
N968	0.021	0.021	0.030
N977	0.013	0.037	0.045
B14	0.014	0.033	0.031
Sample diluent	0.011	0.023	0.027

@: Anti-HPV positive samples diluted with sample diluent.
#: Absorbance at 492nm.

EXAMPLE 7

ASSAYS USING AN αFETOPROTEIN-HCV ANTIGEN COMPLEX FOR THE DETECTION OF ANTIBODIES SPECIFIC FOR HCV

An ELISA was performed as set forth in Example 2, except that an α-fetoprotein (AFP), purified from human cord blood using a monoclonal anti-AFP affinity column, was mixed with the core-env fusion protein of Hepatitis C Virus (HCV) (EN-80-2 antigen, U.S. Application Serial No. 08/447,276) to provide a complex of the AFP and the HCV antigen.

The samples for the assay were as follows. Anti-HCV positive samples were N49, N451, and N103. Anti-HCV negative samples were N949 and N950.

Table 18

Samples	AFP	EN-80-2	AFP & EN-80-2
N8/25X @	0.006 #	0.967	1.497
/50X	0.006	0.623	0.995
/100X	0.010	0.238	0.414
N451/27@x	0.006	1.903	> 2.0
/81x	0.003	0.613	1.219
/243x	0.000	0.190	0.272
N949	0.057	0.041	0.059
N49/27x	0.009	1.055	1.839
/81x	0.004	0.303	0.424
/243x	0.003	0.085	0.116
N103/27x	0.011	> 2.0	> 2.0
/81x	0.001	0.593	0.424
/243x	0.003	0.185	0.116
N950	0.053	0.030	0.058

@: Anti-HCV positive samples diluted with sample diluent.
#: Absorbance at 492 nm.

EXAMPLE 8

THE DETECTION OF MOUSE ANTI-HCV ANTIBODIES INDUCED USING MSA AND AN HCV ANTIGEN

Two immunogens were prepared as follows. The 02 immunogen was prepared by dialyzing a core-envelope fusion antigen from HCV (EN-80-2) against PBS. After dialysis, the antigen was used to immunize ICR mice. The M+02 immunogen was prepared by mixing the core-envelope fusion antigen from HCV (EN-80-2) with mouse serum albumin (MSA) in TNEU buffer for one hour. After mixing, the complex was dialyzed against PBS. Then, the complex was used to immunize ICR mice.

The 02 and M+02 immunogens were administered to ICR mice at 6-8 weeks of age. The first administration, boost and sampling schedule was as follows:

Negative control (ID No. NC):

Day 0, 13 and 28: no immunization or boost.

Day 36: bleeding.

Test group 1 (ID nos. 1-1 and 1-2):

Day 0: 50 μg/mouse of 02 immunogen using complete Freund's adjuvant (CFA), GIBCO, Gaithersburg, MD, USA, 20877.

Day 13: 1st boost: 50 μg/mouse of 02 immunogen using incomplete Freund's adjuvant (IFA), GIBCO.

Day 28 2nd boost: 50 μg/mouse of 02 immunogen in PBS.

Day 36: bleeding.

Test group 2 (ID nos. 2-1 and 2-2):

Day 0: 100 μg of M + 02 immunogen (50 μg of EN-80-2 antigen complexed with 50 μg of MSA) per mouse using complete Freund's adjuvant (GIBCO).

Day 13: 1st boost: 100 μg/mouse of M+02 immunogen using incomplete Freund's

adjuvant (GIBCO).

Day 28 2nd boost: 100 µg/mouse of M+02 immunogen in PBS.

Day 36: bleeding.

The resulting samples were assayed for the presence of antibodies against HCV using methods described above. The assay antigens were MSA and EN-80-2, which had been allowed to form an aggregate in TNEU coating buffer. Rat anti-mouse:HRPO conjugate was used for detection in this assay.

The data in Table 19 showed the presence of an immune response in the test animals. The immune response of immunogen M+02 was better than that of immunogen 02.

Table 19

Samples	MSA	EN-80-2	MSA+EN-80-2
1-1/200X @	0.087 #	0.799	0.929
/400X	0.071	0.367	0.479
/500X	0.069	0.293	0.397
1-2/200X	0.088	1.268	1.466
/400X	0.064	0.627	0.793
/500X	0.072	0.556	0.705
2-1/4000X	0.061	1.457	1.612
/8000X	0.052	0.701	0.843
/16000X	0.072	0.339	0.475
2-2/500X	0.070	> 2.0	> 2.0
/1000X	0.066	0.973	1.122
/2000X	0.087	0.503	0.662
NC/100X	0.117	0.072	0.174
/500X	0.068	0.022	0.122

@: ICR mouse serum diluted with 40% FCS in Tris-HCl buffer.

#: Absorbance at 492nm.

EXAMPLE 9

ASSAYS FOR THE DETECTION IgM ANTIBODIES SPECIFIC FOR HUMAN SERUM ALBUMIN

ELISA's similar to those set forth in Example 1 were used to test samples from HCV positive, HCV negative patients, and HBV positive/HCV negative patients. The ELISA's were performed as set forth in Example 1, with the following changes: The conjugate was a monclonal anti-hIgM:HRPO conjugate, which had been purified by conjugating anti-human immunoglobulin M (anti-hIgM) with horse radish peroxidase (HRPO) using $NaIO_4$ to obtain the anti-IgM:HRPO conjugate. The conjugate was purified by S-200 gel filtration and was diluted in a sample diluent (0.1M Tris-HCl pH: 7.4±0.2, 40% NBBS, 1% BSA, 2% mouse serum). The procedure was modified in that the samples were diluted by putting 20 µl of sample into a test tube, and then adding 1.0 ml of sample diluent (0.1M Tris-HCl ph: 7.4±0.2, 40% NBBS, 1% BSA and 2% mouse serum). Next, 200 µl of the sample diluent and 10 µl of the diluted sample were added into each well of a treated microtiter plate, with some wells retained as blanks. In part (D) procedure of Example 1, 100µl of anti-human IgM:HRPO conjugate solution was added to each well instead of the anti-human IgG:HRPO conjugate solution.

The results of the assay were then evaluated as set forth in paragraph (E) of Example 1 to provide the results set forth in Table 20.

Table 20

Samples	Anti-HCV	Anti-HSA (IgG)	Anti-HSA (IgM)
N1098	- @	0.012,$-	0.060, -
N1032	+ #	> 2.0, +	0.057 -
N329	+	0.251 -	0.664 +

@: non-reactive
#: absorbance at 492nm
$: reactive.

Table 20 indicates that anti-HSA IgM antibody was found in anti-HCV positive serum N329.

EXAMPLE 10

ASSAY FOR THE DETECTION OF IGG ANTIBODIES SPECIFIC FOR HUMAN SERUM ALBUMIN IN SAMPLES THAT ARE HBV POSITIVE AND HCV NEGATIVE

An assay was performed as described above in Example 1, except that the samples were all HBV (HBsAg) positive and HCV negative. The results are set forth in Table 21.

Table 21

Samples	HBsAg	Anti-HCV	Anti-HSA
N1050	+$	-@	0.542, #+
N1052	+	-	0.401, +
N1058	+	-	0.441, +
N1083	+	-	0.355, +
N1087	+	-	0.496, +
K1253	-	-	0.029, -
K1254	-	-	0.039, -
K1255	-	-	0.012, -

$: reactive
@: non-reactive
#: absorbance at 492nm

Table 21 indicates that IgG anti-HSA antibody was found in HBV positive, HCV negative samples.

EXAMPLE 11

ASSAY FOR THE DETECTION OF IGG ANTIBODIES SPECIFIC FOR HUMAN SERUM ALBUMIN IN RANDOM HUMAN SERUM SAMPLES

Four assays in accordance with the assay set forth in Example 1 were performed on random human serum samples. The samples comprised the following groups:

Group 1: Anti-HCV positive, HBsAg positive ..... 2 samples
Group 2: Anti-HCV positive, HBsAg negative ..... 26 samples
Group 3: Anti-HCV negative, HBsAg positive ..... 81 samples
Group 4: Anti HCV negative, HBsAg negative..... 675 samples

The results are set forth in Table 22

Table 22

Group No.	Anti-HSA positive	Anti-HSA negative
1 (2 samples)	2	0
2 (26 samples)	20	6
3 (81 samples)	2	79
4 (675 samples)	11	664

The 11 samples that were anti-HSA positive (*i.e.*, Group No. 4 in Table 22) then analyzed for their absorbance at 492 nm. This is shown in Table 23.

Table 23

Samples	HBsAg	Anti-HCV	Anti-HSA
K159	- @	-	0.459 # + $
K162	-	-	0.367 +
K200	-	-	0.629 +
K326	-	-	0.360 +
K349	-	-	0.703 +
K351	-	-	0.386 +
K458	-	-	> 2.0 +
K560	-	-	0.351 +
K594	-	-	0.676 +
K694	-	-	1.575 +
K749	-	-	0.339 +
K750	-	-	0.058 -
K751	-	-	0.020 -

@: non-reactive.

#: absorbance at 492nm.
$: reactive.

These results indicate that anti-HSA antibodies are more likely to be induced by HCV than by HBV. It appears that each of the viruses induces anti-HSA antibodies having slightly different specificity and/or affinity for HSA.

EXAMPLE 12

ASSAY FOR THE DETECTION IgG ANTIBODY IN A HBV POSITIVE, HCV NEGATIVE SAMPLE

An assay in accordance with the assays set forth in Example 1 was performed on sample N1029, which is HBV positive and HCV negative. The IgG for the assay was human and purified from sample N1029 by using 33% saturated ammonium sulfate and a PROTEIN A affinity column (Pharmacia Biotech). The results are set forth below in Table 24.

Table 24

Sample	EverNew Anti-HCV	Anti-HSA
N1029	0.044 @ - #	0.764 + $
N1029 IgG	0.068 -	0.621 +
Neg. control	0.009	0.020
Pos. control	1.180	1.895
cutoff value	0.186	0.305

@: absorbance at 492nm.
#: non-reactive.
$: reactive.

The results in Table 24 indicate that the anti-HSA that was found in sample N1029 comprises at least IgG, and therefore that IgG anti-HSA can be obtained from an anti-HCV negative, anti-HSA positive sample.

EXAMPLE 13

REGION OF HOMOLOGY BETWEEN HCV CORE PROTEIN AND HUMAN SERUM ALBUMIN

The amino acid sequence of HCV set forth in Muller et al., *J. Med. Virol.* *40*:291-306, 1993, was compared with the immunoassay sequence of HSA set forth in Dugaiczyk et al., *Proc. Natl. Acad. Sci., U.S.A.* *79*(1):71-75, 1982, for regions of homology. As set forth below in Table 25, identical amino acid residues were found from amino acids 116-122 of the HCV core region and amino acids 451-457 of human serum albumin.

Table 25

HCV core region	114	R S R N L G K V I (SEQ ID NO:___)	124
Human serum albumin	449	V * * * * * * * G (SEQ ID NO.___)	459

EXAMPLE 14

THE COOPERATIVE INTERACTIONS BETWEEN SYNTHETIC PEPTIDES AND HTLV-1 ENV PROTEIN

An assay was performed as set forth in Example 4, except that an antigen from the HTLV-1 envelope region was mixed with a synthetic peptide in TNEU buffer to provide a complex of the synthetic peptide and the HTLV-1 env antigen. The sequences of synthetic peptides were as follows.

SP1: NH2-VEVSRSLGKVGTR-COOH (SEQ ID No._____), derived from Bovine serum albumin.

SP2: NH2-VEVSRNLGKVGSK-COOH (SEQ ID No._____), derived from Human serum albumin.

SP3: NH2-VEAARNLGRVGTK-COOH (SEQ ID No._____), derived from Rat serum albumin.

SP4: NH2-RRRSRNLGKVIDT-COOH (SEQ ID No._____), derived from HCV serum albumin.

Human serum albumin was also used in this assay as a control. The samples for the assay were as follows: Anti-HTLV-1 positive samples were SP and POOL. Anti-HTLV-1 negative samples were N522 and N530.

Table 26

Sample	SP1	SP1+ H1	SP2	SP2+H1	SP3	SP3+ H1	SP4	SP4+ H1	HSA	HSA +H1	H1
POOL 50X@	0.010#	0.402	0.000	0.394	0.000	0.601	0.004	0.557	0.001	1.028	0.307
SP 20X	0.000	0.727	0.000	0.779	0.000	1.069	0.002	1.286	0.000	>2.0	0.652
SP 40X	0.000	0.321	0.000	0.410	0.001	0.484	0.000	0.546	0.000	1.048	0.339
SP 80x	0.001	0.155	0.000	0.222	0.003	0.220	0.005	0.235	0.002	0.419	0.165
N522	0.006	0.013	0.013	0.014	0.016	0.011	0.012	0.012	0.013	0.011	0.010
N530	0.010	0.007	0.010	0.014	0.022	0.010	0.012	0.011	0.014	0.009	0.010

#: Absorbance at 492 nm.
@: Anti-HTLV-I positive samples diluted with sample diluent.

These data indicate that synthetic peptides derived from Rat Serum Abumin and HCV core region cooperatively interact with the HTLV-1 env antigen.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

1. An assay for the detection of an IgM antibody against a target antigen in a sample, comprising:

(a) providing a target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex;

(b) contacting the target antigen-serum albumin complex with the sample under conditions suitable and for a time sufficient for the target antigen-serum albumin complex to bind to one or more antibodies for the target antigen present in the sample, to provide an IgM antibody-bound complex; and

(c) detecting the IgM antibody-bound complex, and therefrom determining whether the sample contains the IgM antibody for the target antigen.

2. The assay of claim 1, wherein a second target antigen is complexed with the target antigen-serum albumin complex, to provide a multiple target antigen-serum albumin complex.

3. The assay of claim 1, further comprising the step of binding the target antigen-serum albumin complex to a solid substrate.

4. The assay of claim 1 wherein the serum albumin is selected from the group consisting of human serum albumin, α-fetoprotein, bovine serum albumin, fetal bovine serum albumin, new born bovine serum albumin and mouse serum albumin.

5. The assay of claim 4 wherein the serum albumin is human serum albumin.

6. The assay of claim 1 wherein the sample is an unpurified sample.

7. The assay of claim 6, further comprising, prior to the step of contacting, isolating the sample from an animal.

8. The assay of claim 7 wherein the animal is a human being.

9. The assay of claim 7 wherein the sample is a blood sample.

10. The assay of claim 1 wherein the serum albumin is selected from the same species as the sample.

11. The assay of claim 1 wherein the assay is selected from the group consisting of a countercurrent immuno-electrophoresis (CIEP) assay, a radioimmunoassay, a western blot assay, a radioimmunoprecipitation, an enzyme-linked immuno-sorbent assay (ELISA), a dot blot assay, an inhibition or competition assay, a sandwich assay, an immunostick (dip-stick) assay, a simultaneous assay, an immunochromatographic assay, an immunofiltration assay, a latex bead agglutination assay, an immunofluorescent assay, a biosensor assay, and a low-light detection assay.

12. A method of producing an IgM antibody specific for a target antigen, comprising the following steps:

(a) administering to an animal the target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex under conditions suitable and for a time sufficient to induce the production in the animal of antibodies to the target antigen; and

(b) isolating the antibodies to the target antigen.

13. An assay for the detection of a target antigen in a sample, comprising:

(a) contacting the sample with an IgM antibody produced according to the method of claim 12 under conditions suitable and for a time sufficient for the IgM antibody to bind the target antigen, to provide a bound IgM antibody; and

(b) detecting the bound IgM antibody, and therefrom determining whether the sample contains the target antigen.

14. An assay for the detection of an IgM antibody specific for a serum albumin in a sample, comprising:

(a) contacting the serum albumin with the sample under conditions suitable and for a time sufficient for the serum albumin to bind to one or more IgM antibodies specific for the serum albumin present in the sample, to provide an IgM antibody-bound serum albumin; and

(b) detecting the IgM antibody-bound serum albumin, and therefrom determining whether the sample contains the IgM antibody specific for the serum albumin.

15. The assay of claim 14, further comprising the step of binding the serum albumin to a solid substrate.

16. The assay of claim 14 wherein the serum albumin is selected from the group consisting of human serum albumin, α-fetoprotein, bovine serum albumin, new born bovine serum albumin and mouse serum albumin.

17. The assay of claim 16 wherein the serum albumin is human serum albumin.

18. The assay of claim 14 wherein the sample is an unpurified sample.

19. The assay of claim 18, further comprising, prior to the contacting, the step of isolating the sample from an animal.

20. The assay of claim 19 wherein the animal is a human being.

21. The assay of claim 19 wherein the sample is a blood sample.

22. The assay of claim 14 wherein the assay is selected from the group consisting of a countercurrent immuno-electrophoresis (CIEP) assay, a radioimmunoassay, a western blot assay, a radioimmunoprecipitation, an enzyme-linked immuno-sorbent assay (ELISA), a dot blot assay, an inhibition or competition assay, a sandwich assay, an

immunostick (dip-stick) assay, a simultaneous assay, an immunochromatographic assay, an immunofiltration assay, a latex bead agglutination assay, an immunofluorescent assay, a biosensor assay, and a low-light detection assay.

23. A method of producing an IgM antibody against a serum albumin, comprising the following steps:

(a) administering to an animal a target antigen-serum albumin complex, comprising the target antigen complexed with a serum albumin, under conditions suitable and for a time sufficient to induce the production in the animal of antibodies specific to the serum albumin; and

(b) isolating the antibodies to the serum albumin.

24. The method of claim 23 wherein the method further comprises the step of isolating the IgM antibody from the animal.

25. A method for the detection of a serum albumin in a sample, comprising:

(a) contacting the sample with an IgM antibody produced according to the method of claim 23 under conditions suitable and for a time sufficient for the IgM antibody to bind the serum albumin, to provide a bound IgM antibody; and

(b) detecting the bound IgM antibody , and therefrom determining whether the sample contains the serum albumin.

26. A method for the isolation of a serum albumin from a sample, comprising:

(a) contacting the sample with an IgM antibody produced according to the method of claim 23 under conditions suitable and for a time sufficient for the IgM antibody to bind the serum albumin, to provide bound serum albumin; and

(b) isolating the bound serum albumin from the sample.

27. A method of producing an assay to detect an IgM antibody against a target antigen comprising contacting the target antigen with a serum albumin under conditions suitable and for a time sufficient for the target antigen to complex with the serum albumin to form a complex, and then binding the complex to a solid substrate.

28. The method of claim 27 wherein the target antigen is contacted with the serum albumin in a TNSCN buffer.

29. A composition capable of binding to an IgM antibody against a target antigen, the composition comprising an isolated target antigen complexed with a serum albumin to provide an isolated target antigen-serum albumin complex.

30. The composition of claim 31 wherein the isolated target antigen-serum albumin complex is bound to a solid substrate.

31. A composition capable of binding to a target antigen, the composition comprising an IgM antibody produced against a target antigen-serum albumin complex comprised of the target antigen complexed with a serum albumin, the IgM antibody bound to a solid substrate.

32. A kit for the detection of a target antigen, comprising:

(a) the target antigen complexed with a serum albumin to provide a target antigen-serum albumin complex; and

(b) one or both of a reagent or a device for detecting the complex or an IgM antibody bound to the complex.

33. A kit for the detection of a target antigen:

(a) an IgM antibody produced according to the method of claim 12; and

(b) one or both of a reagent or a device for detecting the IgM antibody.

INTERNATIONAL SEARCH REPORT

Inter. ...onal Application No
PCT/US 97/18194

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543 G01N33/532 G01N33/58 G01N33/569 G01N33/68 G01N33/576

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D.C. PHIPPS ET AL.: "An ELISA employing a Haemophilus influenzae type b oligosaccharide-human serum albumin conjugate correlates with the radioantigen binding assy." JOURNAL OF IMMUNOLOGICAL METHODS, vol. 135, no. 1-2, 1990, AMSTERDAM NL, pages 121-128, XP002055506	1,3-11, 29-31
A	see page 122, column 1, line 25 - page 123, column 2, line 3; figure 4	2,12-28, 32,33
A	PATENT ABSTRACTS OF JAPAN vol. 15, no. 391 (P-1259), 3 October 1991 & JP 03 156369 A (M. KOICHI ET AL.) see abstract	25-28

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
12 February 1998	26/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Bohemen, C

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INTERNATIONAL SEARCH REPORT

Inter. onal Application No
PCT/US 97/18194

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 120, no. 25, 20 June 1994 Columbus, Ohio, US; abstract no. 321040, XP002055507	12,13
A	see abstract & N. KOBAYASHI ET AL. : "Specificity of the polyclonal antibodies raised against a novel 25-hydroxyvitamin d3-bovine serum albimin conjugate linked through the c-11 alpha position." JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 48, no. 5-6, 1994, NEW YORK NY USA, pages 567-572,	14-24

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